ERRATUM

In articles 7231, 7385 and 7404, *Microsporon apiospormum* should read *Monosporum apiospermum.*

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7425 C*

Utilization of Free and Acetylated l- and dl-Tryptophane by Oidium Lactis.

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It has long been known that *Oidium lactis* is able to utilize the nitrogen of several natural amino acids for growth when such acids serve as the sole source of nitrogen. Under such circumstances, *l*-tryptophane is converted into *l*-indolelactic acid.[1] In conjunction with a series of studies on tryptophane metabolism recorded elsewhere, in which *Oidium lactis* was used to synthesize indolelactic acid,[2] it seemed of interest to compare the availability of *l*- and *dl*-tryptophane and acetyl-*l*- and acetyl-*dl*-tryptophane as sources of nitrogen for the growth of this mold.

Accordingly, 8 liters of a synthetic medium containing 80.0 gm. invert sugar, 8.00 gm. K_2HPO_4, 0.800 gm. $MgSO_4$, and traces of NaCl and $FeCl_3$ were prepared and divided equally among eight 2800 cc. culture flasks. To each of one pair of the flasks were added 2.000 gm. of *l*-tryptophane; to each of a second pair, 2.000 gm. of *dl*-tryptophane; to each of a third pair, 2.412 gm. of acetyl-*l*-tryptophane; and to each of the fourth pair, 2.412 gm. of acetyl-*dl*-tryptophane. The flasks were fitted with cotton plugs and sterilized. A 10 cc. aliquot was withdrawn from each for initial N analysis (by micro-Kjeldahl). To each flask were then added equal volumes of a suspension of *Oidium lactis* (American Type Culture Collection, No. 4798). The culture media were incubated at 20°C. for 4 weeks, at the end of which time they were sterilized. N determinations were again made on aliquots of each of the mycelium-free filtrates. The indolelactic acid was isolated from the *l*- and *dl*-tryptophane media according to the method of Ehrlich and Jacobsen[1] and the tryptophane was obtained from the residues by isolation through the mercuric sulfate salt, as in its preparation from protein.[3] No significant changes in N content having been observed in the acetyl-tryptophane media, no attempts were made to isolate indolelactic acid.

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* P represents a preliminary, C a complete manuscript.

[1] Ehrlich, F., and Jacobsen, K. A., *Ber.*, 1911, **44**, 888.

[2] Bauguess, L. C., and Berg, C. P., *J. Biol. Chem.*, 1934, **104**, 675.

Results of the *l*- and *dl*-tryptophane studies appear in Table I. The final N contents of the mycelium-free *l*- and *dl*-tryptophane media did not differ appreciably. The tryptophane isolated from the *dl*-tryptophane flasks was strongly dextro-rotatory, indicating that the *l*-tryptophane was utilized preferentially. The specific rotation of the tryptophane in the *l*-tryptophane flasks had not changed. The methods of isolating the tryptophane and the indolelactic acid are not to be regarded as quantitative. The amounts isolated, in each case, account for approximately 70% of the residual N. *d*-Tryptophane apparently does not inhibit the utilization of N by the mold. In each instance, as in previous studies,[2] the indolelactic acid isolated possessed the same optical properties, whether isolated from the *l*- or from the *dl*-tryptophane flasks. There is some doubt as to whether enough of the *d*-tryptophane was utilized to warrant con-

TABLE I.

Tryptophane in Culture Media	Tryptophane Isolated		Indolelactic Acid Isolated		N Content of Mycelium-free Media	
gm.	gm.	$[\alpha]_D^{20°}$, 0.5 % in water	gm.	$[\alpha]_D^{20°}$, 1.31 % in water	Initial mg..	Final mg.
2.0 (*l*)	0.390	—32.8	0.84	—5.2	275.6	153.8
2.0 (*l*)	0.384	—33.0	0.80	—5.2	275.6	152.9
2.0 (*dl*)	0.320	+21.8	1.01	—5.2	276.0	158.4
2.0 (*dl*)	0.323	+22.0	0.95	—5.2	276.0	158.0

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[3] Hopkins, F. G., and Cole, S. W., *J. Physiol.*, 1901, **27**, 418; Cox, G. J., and King, H., *Organic Syntheses*, 1930, **10**, 100.

cluding definitely that this isomer is converted into *l*-indolelactic acid, as the data suggest. A test of *d*-tryptophane alone is contemplated to clear up this point.

Only traces of mycelium were obtained in the acetyl-*l*- and acetyl-*dl*-tryptophane culture flasks. N analyses of the mycelium-free cultures showed that their total N content (275.0 mg.) had not decreased during the period of incubation. Apparently, *Oidium lactis* is unable to hydrolyze either acetyl-*d*- or acetyl-*l*-tryptophane. This is in striking contrast to the utilization of acetyl-*l*-tryptophane by the rat.[4]

7426 C

Effect of Thyroparathyroidectomy and Thyroxin on Rate of Atrophy of Skeletal Muscle.

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Previous investigations have indicated that the thyroid secretion exerts an influence on skeletal muscle. Simpson[1] suggested that the lack of development of skeletal muscle in cretin sheep might be due to inactivity rather than a specific action of thyroid secretion on muscle growth. Gudernatsch[2] showed that the feeding of thyroid material caused an accelerated rate of atrophy of the tadpole's tail during metamorphosis. This effect has usually been attributed to the mechanical influences of increased growth changes rather than a specific effect of thyroid substance on autolysis.[3]

This report is concerned with the influence of thyroparathyroidectomy and thyroxin administration on the rate of atrophy of the denervated gastrocnemius muscle of the rat. We have previously reported[4] some effects of denervation on rat's skeletal muscle and described the technique employed. The weight of the denervated muscle has been compared to that of the opposite control muscle in a series of adult animals 7, 14, 21 and 28 days after section of the sciatic nerve. A series of 26 animals were subjected to thyroparathyroidectomy a few weeks prior to the time of nerve section. A group of 36 animals were given subcutaneous injections of *dl*-thyroxin. The amounts given totaled approximately 1 mg. per rat per week, and the injections were made at intervals of 3 to 4 days. The initial dose was given 3 to 4 days prior to denervation of the muscle. The effectiveness of the thyroxin administration was determined by frequent measurements of the oxygen consumption of the animals. The amount of thyroxin given was adequate to cause an elevation of the metabolic rate but less than that required to cause an appreciable loss of body weight. In order to measure the rate of atrophy it is essential that the control muscle should neither gain nor loose weight subsequent to the time of denervation of the opposite muscle. A group of animals from the same stock served as controls for the above experiments.

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[4] Berg, C. P., Rose, W. C., and Marvel, C. S., *J. Biol. Chem.*, 1929, **85**, 207; du Vigneaud, V., Sealock, R. R., and Van Etten, C., *J. Biol. Chem.*, 1932, **98**, 565; Berg, C. P., *J. Biol. Chem.*, 1934, **104**, 373.

[1] Simpson, E. D., *Am. J. Physiol.*, 1927, **80**, 735.

[2] Gudernatsch, J., *Am. J. Anat.*, 1914, **15**, 431.

[3] Bradley, H. C., *Physiol. Rev.*, 1922, **2**, 415.

[4] Hines, H. M., and Knowlton, G. C., *Am. J. Physiol.*, 1933, **104**, 379.

The average values for the loss of weight by the denervated muscle in the 3 groups of animals are given in Table I. Studies made on the water content of the muscles indicate that no significant difference existed between the water content of muscles from animals in the various groups. These results indicate that denervated muscle in animals subjected to thyroparathyroidectomy loses weight at a slower rate than does denervated muscle in control animals selected from the same stock and kept on the same diet. The experiments demonstrate that thyroxin administration accelerates the rate of atrophy of denervated muscle. It is believed that thyroxin exerts this effect by acting as an accelerator to the katabolic processes involved in the disintegration of the cytoplasm of the denervated muscle. These results also indicate that thyroxin is active in skeletal muscle deprived of a nerve supply and offer additional evidence of a direct influence of this hormone on muscle cells.

TABLE I.

Summary of % of Weight Lost by the Denervated Muscle.

Days after Denervation	Control Animals	Thyroparathyroidectomized Animals	Animals receiving Thyroxin
7	22.3	21.5	33.1
14	48.3	43.1	58.7
21	61.9	53.8	71.8
28	72.3	66.8	76.6

7427 C

Methylene Blue in Illuminating Gas Poisoning.

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Current reports[1] regarding the action of methylene blue in illuminating gas poisoning are sufficiently contradictory to warrant further experimental investigation of its value. Determination of prophylactic action is one method of approach.

In the experiments reported below the protective action of methylene blue in asphyxiation by illuminating gas was measured by determining the comparative lethal interval in 3 series of methylene blue injected as compared to non-injected white rats. In all cases reported, litter mates of similar weight and sex were compared; the injections were made intraperitoneally, about ½ hour previous to asphyxiation. When 50 mg. M. B. per kg. of body weight were injected it could be seen within 15 minutes in the capillaries of non-hairy regions of the body and in the conjunctiva. Usually 5 mg. (1 cc. aq. soln.) per kg. were used but varying amounts, between 5 and 50 mg. were tried, no advantages being observed with the larger amounts. Control animals received a similar amount of Ringer's solution.

In series 1, injected and non-injected normal litter mates were compared. In series 2, normal rats were compared to litter mates in which the basal metabolic rate had been increased by feeding ½ gr. U.S.P. desiccated thyroid daily for 15 consecutive days preceding asphyxiation. In series 3, normal rats were compared to litter mates in which the B.M.R. had been increased by injection of 1 cc. of 0.1% alpha dinitrophenol [(1-2-4) ($C_6H_3(NO_2)OH$)] intraperitoneally ½ hr. previous to asphyxiation or by giving the same amount by stomach tube on 3 successive days previous to asphyxiation.

The gas used was introduced directly from the city main into a closed circuit respiration apparatus equipped with a blower, soda lime container, and glass animal chamber. A 1.89% gas-air mixture (0.36% CO) was used. Cessation of respiratory movements served as the criterion of death in all cases.

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* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Bussabarger, Robert A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 598.

RESULTS.

Series 1. Normal Animals. (Age about 60 days.)

No. of animals		Mean lethal interval, min.			
Control	M. B. injected*	Control	M. B. injected	Mean diff.	Standard deviation of mean diff.
9	10	23.2	26.5	3.3	2.5
Series 2. Increased Basal Metabolic Rate—Thyroid Fed.					
Control	Thyroid fed	Control	Thyroid fed		
10	9	30.5	15.5	15	5.4
Thyroid fed	Thyroid fed and injected with M. B.	Thyroid fed	Thyroid fed and injected with M. B.		
9	11	15.5	9.7	5.8	3.6
Series 3. Increased Basal Metabolic Rate—Dinitrophenol Injected. (Age about 200 days.)					
Control	M. B. inj.	Control	M. B. inj.		
11	8	42.1	39.2	2.9	6.2
Control	M. B. and D. N. T. injected	Control	M. B. and D. N. T. injected		
11	15	42.1	26.5	15.6	5.8
Control	D. N. T. injected†	Control	D. N. T. injected		
11	17	42.1	23.9	18.2	5.8
D. N. T. inj.	D. N. T. and M. B. inj.	D. N. T. inj.	D. N. T. and M. B. inj.		
17	15	23.9	26.5	2.6	4.1

* Methylene Blue injected.
† Dinitrophenol "

Summary and Conclusions. The data reported include the figures obtained in study of 100 animals. Many more were sacrificed in trial procedures. The results show no significant action by methylene blue as a prophylactic agent in illuminating gas poisoning. The very greatly decreased lethal interval in animals receiving desiccated thyroid or dinitrophenol is worthy of note.

7428 C

Measurement of Daily Heat Production of Albino Rat from the Insensible Water Loss.

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A method is needed for measuring the daily energy requirements of the albino rat. The 24-hour insensible loss of weight and the percentage of the total heat loss that was due to vaporization of water have been measured to ascertain whether or not the measurement of the imperceptible water loss would fulfill this need.

Johnston and Newburgh[1] have applied the method in man. Each rat was placed in a wire cage suspended above a porcelain pan in which the excreta was collected under mineral oil. The weighings were made every 24 to 48 hours on a balance accurate to 200 mg. The weights of the food, water and excreta were obtained from the difference in weight of the container and contents at the beginning and end of the period. The vaporization of water, from the mouth of the water container, averaged 1 gm. per day as measured by a control. The diet of casein 20%, corn starch 48%, lard 16%, cod liver oil 3%, salt mixture (Osborne and Mendell) 5%, dried yeast 8% contained 3.7% moisture but did not lose water at room temperature. The rats' 24-hour excreta under oil maintained a constant weight for a week. The temperature of the laboratory varied from 23 to 27°C. Changes in barometric pressure and humidity had no significant influence.

Three grown male rats were studied. The daily food consumption yielded 43.5, 34.6 and 29.7 calories for periods of 42, 29 and 24 days with changes in body weight of only 1.6, 0.5 and 1.0 gm. respectively. In calculating the caloric value of the diet the values 4,4 and 9 were used for carbohydrate, protein and fat.

The vaporization of water is the only factor in the insensible loss of weight that removes heat from the body and was calculated by the formula of Isenschmid[2]: Ins. L. Wt. = $H_2O + (CO_2–O_2)$. The carbon dioxide excreted and oxygen absorbed were calculated from the metabolic mixture by the constants for protein, carbohydrate and fat given by Newburgh, Johnston and Falcon-Lesses.[3] The metabolic mixture was the same as the food ingested, since the animals were in nitrogen balance, and the weight remained nearly constant. The vaporization of one gram of water from the surface of a mammal requires 0.58 calorie. The heat lost by evaporation of water was compared with the caloric value of the metabolic mixture.

The calculations were by the following formula:

(Wt. rat beginning period + water + food) — (Wt. rat end period + excreta = Ins. L. Wt. H_2O = Ins. L. Wt. — $(CO_2—O_2)$. $H_2O \times 0.58$ = calories lost by vaporization of water.

The averages of the results for each animal are shown in Table I.

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[1] Johnston, Margaret W., and Newburgh, L. H., *J. Clin. Invest.*, 1930, **8**, 147.

[2] Isenschmid, *Med. Klin.*, 1918, **14**, 1128.

[3] Newburgh, L. H., Johnston, M. W., and Falcon-Lesses, M., *J. Clin. Invest.*, 1930, **8**, 161.

The daily variations in the insensible loss of weight of each rat were probably due to activity which was not measured, but when periods of 4 to 10 days are compared the variations are small (Table II). An average of 21.3% of the total heat loss was due to imperceptible water. This compares favorably with figures obtained for the rat in the basal state by Greene and Luce.[4] When the average heat lost by the evaporation of water for periods of 4 to 10 days is considered to be 21.3% of the total as calculated from the metabolic mixture, the variations are no greater than those found in measuring the basal metabolism (Table II).

TABLE I.

	Average daily				
Rat	Ins. L. Wt.	Ins. water	Calories lost by Ins. water	Daily caloric value of metabolic mixture	Average % total heat lost by Ins. water
1	18.8	16.3	9.4	43.5	21.6
2	13.9	12.4	7.2	34.6	20.8
3	12.3	11.0	6.4	29.7	21.5
				Ave.	21.3

TABLE II.

Rat	No. days in period	Ave. Ins. L. Wt.	Ave. daily heat production from Ins. water	Calories from metabolic mixt. daily	% variation
1	7	18.3	42.8	43.5	—1.6
	6	19.8	46.8	43.5	+7.1
	9	18.8	44.2	43.5	+1.6
	4	17.8	41.8	43.5	—3.9
2	7	13.9	33.8	34.6	—2.3
	7	14.5	35.3	34.6	+2.0
	7	13.7	33.4	34.6	—3.5
	7	13.9	33.9	34.6	—2.3
	7	14.1	34.1	34.6	—1.4
	7	13.5	32.9	34.6	—4.9
	4	13.1	32.0	34.6	—7.5
3	7	12.0	29.1	29.7	—2.0
	7	14.0	34.1	29.7	+14.8
	7	12.5	30.0	29.7	+1.0
	10	12.3	30.0	29.7	+1.0

Summary. I. A method for measurement of the 24 hourly insensible loss of weight of the albino rat has been described. II. The results have shown that the daily heat production of the albino rat can be accurately determined from the weight of the insensible water loss. III. The heat lost by vaporization of water averaged 21.3% of the total calculated heat production in an environmental temperature of 23 to 27°C.

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[4] Greene, Jas. A., and Luce, R. P., *J. Nutrition,* 1931, **4,** 371.

7429 C

Absence of Response of Anemia of Myxedema to Liver Extract.

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The clinical manifestations of myxedema are enough like those of pernicious anemia to account for the fact that myxedema is more often mistaken for pernicious anemia than for any other disease. Myxedema may present a yellowish pallor, absence of weight loss, achlorhydria, anemia, color index about unity, parasthesias, difficulty in walking (not true postero-lateral sclerosis), increased serum bilirubin (indirect van den Bergh), leukopenia, urobilinogen in the urine, and remissions. Five cases have been reported in which myxedema and pernicious anemia co-existed in the same patient.[1, 2]

The possibility that there may be a decrease or absence of Castle's factor in the gastric secretion of patients with myxedema led us to determine the response to liver extract therapy of patients with myxedema. Five patients not reported here were similarly studied during 1930 and 1931 by Doctors F. N. Cole and A. S. Fourt in this hospital. No response was obtained in any case but the exact figures are not available.

The initial hemoglobin and erythrocyte levels were not low enough in some of the cases to permit of much increase in reticulocytes. In one case in which the initial hemoglobin value was 54% and the color index was unity, there was a reticulocyte response up to 5.2%, but this response lasted until the 24th day and did not correspond to the usual reticulocyte crisis. After 41 days of treatment the hemoglobin had not increased and the erythrocytes had increased but 500,000 per cu. mm. The patient received thyroid extract during the last days of this period. In the other patients there was neither a reticulocyte increase nor an increase in hemoglobin or erythrocytes.

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[1] Sturgis, C. C., and Isaacs, Raphael, *Proc. Cent. Soc. Clin. Res., J. Clin. Invest.*, 1930, **8**, 663.

[2] Means, J. H., Lerman, J., and Castle, W. B., *New Eng. J. Med.*, 1931, 204.

TABLE I.

Shows the lack of reticulocyte response to liver extract.

Case No.	Before Liver	No. of days after Liver																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	....	1.6	1.0	1.4	1.0	0.6	2.8	....	2.6	....	....	....	....	....	....	....	1.5	....	....	....	....	....	....	....	....
2	0.8	....	1.1	....	1.0	....	1.2	4.1	....	4.0	4.6	....	5.2	3.2	2.6	....	2.6	....	3.0	....	....	5.3	....	....	3.0
3	0.0	0.0	....	....	0.0	0.4	0.4	0.8	0.0	0.0	0.2	0.2	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	....	....
4	0.2	....	0.6	0.6	0.8	0.6	1.0	1.2	1.4	1.2	0.8	1.0	....	0.8	....	1.0	....	....	0.6	....	....	....	....	....	....
5	0.2	....	....	....	0.0	....	0.0	0.4	0.2	0.2	0.0	0.4	0.6	0.0	0.0	0.2	0.0	0.0	0.6	0.6	0.0	0.0	0.0	....	....
6	....	0.4	0.6	0.4	0.5	0.4	0.0	0.5	0.8	0.1	0.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
7	....	0.2	0.2	0.2	0.2	....	0.0	0.0	....	....	0.0	....	2.8	1.6	0.8	0.0	....	....	....	....	....	....	....	....	....
	....	0.6	0.4	0.4	0.8	0.6	....	0.2	0.2	0.6	0.4	....	....	....	....	....	....	....	....	....	....	....	....	....	....
8	....	0.0	0.6	0.2	0.0	....	0.0	....	....	....	0.0	....	0.6	2.2	0.6	1.4	....	....	....	....	....	....	....	....	....
9	1.4	0.8	0.6	1.2	0.6	0.8	1.6	2.0	2.0	0.2	3.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
11*	1.6	2.6	2.4	2.2	2.6	....	1.4	1.6	1.2	1.0	....	1.2	1.2	1.4	0.2	....	....	....	....	....	....	....	....	....	....

* Case 11. Anemia partly due to recent hemorrhage.

TABLE II.
Shows the van den Bergh reaction, free hydrochloric acid in the gastric juice, and the lack of response to liver extract.

Case	Before liver			van den Bergh	Daily liver dosage	Days receiving liver	Free HCl	After liver		
	Hgb.	RBC	Ret. %					Hgb.	RBC	Ret. %
1	83	4.2	1.6	2.0 Ind.	6 vials	10	normal			2.8
2	54	2.7	0.8	neg.	”	41	low after H	55	3.2	5.2
3	68	3.7	0.0	”	”	10	0	54	3.0	0.2
4	75	4.0	0.2	”	”	10	0	83		0.8
5	65	3.6	0.2		”	10	normal	64	3.5	0.6
6	72	3.8	0.4	1.2 Ind.	4 oz.	20		66	3.5	0.0
7	75	3.1	0.2	0.1 ”	3 cc. S.C. every other day	14	0	74	3.4	2.8
	83	3.4	0.6		4 oz.	34		82	3.7	0.8
8	80	4.4	0.0		total 7 doses 3 cc. S.C.	10	normal			0.6
9	82	3.7	1.4		I.M 100 gm.	equivalent of 100 gm. intramuscular for 11 days	low	80	3.4	3.0
10	55	3.5		neg.	6 vials	13		55	2.9	
11*	20	2.5	1.6	”	12 cap Extra.	10	0	30	3.4	1.2

* Case 11. Anemia partly due to recent hemorrhage.

7430 C

Effect of Fluorine on Blood and Respiration.

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The interest of the authors in fluorine dates from the discovery by Ostrem, Nelson, Greenwood, and Wilhelm[1] of fluorine in the drinking waters of communities in the State of Iowa, with the resulting production of mottled enamel of the teeth. Smith, Lantz and Smith[2] have produced considerable evidence that excessive fluorine in water will cause mottled enamel in man. The question arises as to whether fluorine produces other harmful effects in the body.

The following work relates to the effect of fluorine on respiration, blood pressure, hemoglobin, blood calcium, blood phosphorus, and coagulation of blood. Sollmann[3] refers to the work of Tappeiner and states that in mammals NaF causes salivation, gastro-enteritis, dyspnea, muscular weakness and tremors, epileptic convulsions, fall of blood pressure, and stoppage of respiration and heart. Schulz[4] observed similar results. Gerschmann[5] reports that injection of NaF reduces blood calcium. Stuber and Lang[6] observed hemophilic patients with high amounts of F in the blood, and they accept the idea that high F content of blood accompanies a long clotting period. Schwyzer[7] observed a high degree of coagulability of the blood of rabbits, dogs, and pigeons which received F. Hauck, Steenbock, Lowe, and Halpin[8] reported analyses of the calcium and phosphorus of the blood of chickens receiving fluorides, but they admit that their data are insufficient to draw any definite conclusions. Phillips[9] states, "Very little work is reported dealing with the influence of fluorine on blood composition."

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[1] Ostrem, C. T., Nelson, V. E., Greenwood, D. A., and Wilhelm, H. A., *Science*, 1932, **76**, 575.

[2] Smith, M. C., Lantz, E. M., and Smith, H. V., *Ariz. Agr. Exp. Sta. Bull.*, 1931, **32**, 253.

[3] Sollmann, T., A manual of pharmacology, 1932, 898.

Seven dogs, 5 females and 2 males, were employed in studies on respiration and blood pressure. They weighed from 6 to 26.8 kg. The remainder of the experiments were performed on puppies—13 in number, 5 males and 8 females. Nembutal in distilled water was used as the anesthetic. It was administered intraperitoneally at the rate of 30 mg. per kilo body weight. NaF was injected into the femoral vein. Blood pressure records were taken from the central (heart) end of a ligated carotid artery with a mercury manometer writing on a long paper kymograph. Eight per cent citrate solution was used to prevent clots in the cannulae and tubes. Respiratory tracings were made with a Becker air tambour connected to a pneumograph tied around the chest. Newcomer's method was used for hemoglobin determinations. The Brodie-Russell-Boggs coagulometer was used in studying the time of coagulation of blood. Calcium was determined in blood plasma by the Clark and Collip modification of the Kramer Tisdall method and acid soluble inorganic phosphorus of Youngberg's method.

Table I summarizes the results obtained on respiration and blood pressure; the data show that injection of 1.5 to 5.3 mg. of F as NaF per kilo of body weight produces a detectable effect on respiration. The average respirations increased from 15 per minute to 22 per minute, by injecting from 1.5 to 5.3 mg. of F as NaF per

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[4] Schulz, H., *Arch. Exp. Path. v. Pharm.*, 1889, **25**, 326.

[5] Gerschmann, R., *Ann. farm bioquim.*, 1930, **1**, 77.

[6] Stuber, B., and Lang, K., *Biochem. Z.*, 1929, **212**, 96.

[7] Schwyzer, F., *Biochem. Z.*, 1914, **60**, 32.

[8] Hauck, H. M., Steenbock, H., Lowe, J. T., and Halpin, J. G., *Poultry Sci.*, 1933, **12**, 242.

[9] Phillips, P., Thesis, Univ. of Wis., 1933.

TABLE I.
Effect of Intravenous Injection of Fluorine as Sodium Fluoride on Respiration and Blood Pressure.

Dog No.	Respirations per min. before injection of F	Mg. F per kilo body wt. required to produce detectable change in respiration	Respirations per min. after injection of F	Blood pressure in mm. Hg before injection of F	Mg. F per kilo body wt. required to produce detectable change in blood pressure	Blood pressure in mm. Hg after injection of F	Mg. F per kilo body wt. which caused death of animal
1	*			145	31.7	120	92.6
2	12	5.3	16	150	20.4	114	47.5
3	†			200	23.8	164	32.5
4	12	1.5	20	180	22.7	120	30.4
7	22	2.0	32	175	16.0	160	31.9
Averages	15.3	2.9	22.6	170	22.9	135.6	47.0

* Artificial respiration was used during the first part of the experiment.
† Artificial respiration was used throughout experiment.

kilo of body weight. Furthermore, the data reveal that injection of 16 to 31.7 mg. of F as NaF per kilo of body weight causes a perceptible lowering of blood pressure. The average blood pressure was lowered from 170 mm. Hg. pressure to 135.6 mm. Hg. pressure, by increasing the concentration of F as NaF from 0 mg. per kilo body weight to a level of 16 to 31.7 mg. of F as NaF per kilo body weight. The above amounts of NaF were initial doses. At intervals following the initial dose larger amounts of NaF were injected into the femoral vein. Following increased doses, respiration increased to as high as 150 per minute, and blood pressure fell to as low as 40 mm. Hg. In all cases there was respiratory paralysis before stoppage of the heart. Two dogs received orally per kilo of body weight 20 times the amount of F as NaF ordinarily consumed daily by human beings in an area where mottled enamel is endemic, without noticeable effect on respiration and blood pressure. The dogs received orally 4.5 mg. F as NaF per kilo of body weight, which was followed in a few minutes by 9 mg. of F as NaF and then up to higher doses at intervals of a few minutes. When the oral dosage of F reached 22.6 mg. per kilo of body weight, a detectable effect on respiration and blood pressure resulted. At the higher levels vomiting and salivation occurred which prevented accurate determinations. Autopsy of these dogs showed gastroenteritis and congestion of the kidneys. Dr. A. E. Merkel of Ankeny has made blood pressure studies on 65 school children who received fluorine water and exhibited mottled enamel, and he has observed that there is no significant variation in blood pressure from the normal.

Determinations of hemoglobin, coagulation time of blood, blood calcium, and blood phosphorus were made every 2 weeks for 18 weeks on puppies (5 to 9 months old) receiving the following amounts of F in mg. per kilo body weight: 0.0, 0.45, 0.90, 2.26, and 4.52. The average values of hemoglobin, expressed in gm. per 100 cc. of blood, were: 11.86, 12.37, 12.26, 11.96, and 12.18 respectively; and the coagulation times of blood in seconds were: 84.0, 72.5, 85.3, 95.4, and 91.4 respectively. The average values for calcium, expressed as mg. calcium per 100 cc. of blood plasma, were: 12.17, 12.05, 12.0, 12.15, and 12.07; and the results, obtained on acid soluble inorganic phosphorus, expressed as mg. P per 100 cc. blood plasma, were: 5.58, 5.48, 5.29, 5.24, and 5.13 respectively. The fluorine was given orally as NaF.

Southern Section.

Tulane University, May 11, 1934.

7431 P

The Fecundity of Parasitic Female Strongyloides.*

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In human and animal infections with *Strongyloides stercoralis* several undetermined factors operate to produce a variability and instability of the strains of the organism.[1] Possibly the presence of parasitic male worms during the period between adolescence and maturity of the parasitic females may play an important part in determining the type of their progeny.[2] In order to obtain information on the fecundity of the parasitic females, we have inoculated 17 dogs with 5 known human strains of *Strongyloides stercoralis* and a rhesus monkey with a chimpanzee strain. The abdominal skin, the buccal cavity and the intracecal mucosa were used as sites of entry for the infective stage (filariform) larvae. Detailed information was obtained concerning the source, type and number of larvae in each inoculum, the prepatent period, the daily number of *Strongyloides* eggs or larvae recovered from the feces, and, on sacrificing the host, the position, number and condition of the parasitic females. The prepatent periods usually ranged from 11 to 18 days, although one dog was positive on the fifth day, one was not detected until the 143rd day and one apparently never passed larvae in the stools.

The number of eggs produced has been shown to rise rapidly to a maximum, after which it declines to a lower level which is maintained for a time and then is gradually reduced to a base level of zero.

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* Supported by a grant from the Committee on Scientific Research, American Medical Association.

[1] Faust, E. C., and Kagy, E. S., *Am. J. Trop. Med.*, 1932, **13**, 47.

[2] Faust, E. C., *Am. J. Hyg.*, 1933, **18**, 114.

Culture of larvae in the feces, as recommended by Sandground,[3] is a poor index of production of progeny, because so many larvae are passed in a non-viable condition. Likewise, samplings of less than 5 gm. of feces daily are frequently inadequate to detect the larvae present. Even then great care is needed to discover and identify disintegrating larvae, which frequently number 5 to 90% of the total yield.

At the beginning of egg production or soon afterwards the parasitic female worms invade the intestinal mucosa, usually in the region of the duodenum or jejunum, where they deposit their embryonating eggs in the tissues. In the canine hosts the eggs usually hatch in the tissues and the rhabditiform larvae gradually work their way out into the lumen of the bowel, pass down and are evacuated in the feces. In the monkey infection eggs are usually discharged in the unhatched condition. Daily and average weekly counts of progeny in the feces were usually far in excess of uterine eggs in the parasitic females, indicating that several full clutches of eggs were oviposited daily. Furthermore, the yield of parasitic females at autopsy was frequently in excess of expectations, based on the number of infective-stage larvae in the inocula. Twice the number of parasitic females recovered was actually more than the number of larvae in the inocula. This excess can only be explained by the mechanism of internal (hyper-) infection, by which daughter rhabditiform larvae transform into dwarf filariform larvae as they pass down the bowel, thence penetrating the mucosa of the large bowel to effect a migration *via* the blood stream to the lungs and return to the intestine, to develop into and settle down as adult worms usually in the mucosa of the duodenum and jejunum. Likewise, adolescent and recently matured parasitic female worms, found by us in the duodenal and jejunal wall, along with post-productive females, several months after the host has been inoculated, quite likely belong to this type. Many female worms recovered at autopsy were non-fecund. In several instances the feces had yielded no larvae for weeks or months. Nevertheless, these females were still living and became active in physiologic saline solution. Other females were encapsulated; still others were surrounded by phagocytic white cells, which had occasionally broken down the capsules and were attacking the worms. Hence reduction in egg-production and the eventual loss of adult female worms by the host is probably due, not to migration of these worms out of the tissues and their loss in the passed feces, but to host tissue reaction, involving 2 mechanisms, namely, encapsulation and phagocytosis.

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[3] Sandground, J. H., *Am. J. Hyg.*, 1926, **6**, 337.

Although infection of the dog with human strains of *S. stercoralis* is maintained only for a period of weeks or months, in contrast to years in man, the natural host, it is unlikely that man remains infected indefinitely without outside exposure or hyperinfection. Suspected patients should never be pronounced "negative" for *Strongyloides* until prolonged intensive fecal examinations have been made. Even then the patient may be harboring tens or hundreds of post-productive females, which may account for characteristic symptoms of chronic strongyloidosis. Likewise the number of larvae recovered from the feces is not necessarily an index of the degree of infection in the intestinal wall.

7432 P

Filarial Periodicity in the Dog Heartworm, Dirofilaria Immitis, After Blood Transfusion.

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Lane[1, 2] believes that the mechanism of filarial periodicity in *filariasis bancrofti* in man is due to a simultaneous daily parturition of the females, involving the destruction daily of as many microfilariae as are produced. O'Connor[3, 4] has found histological evidence to support Lane's hypothesis, and concludes that this occurs about midday in Puerto Rico. On the other hand, Low and Manson-Bahr[5] criticize the theory, because of the effects of absorption of these millions of dead microfilariae in the body, and because microfilariae have been kept alive outside the host for a week. Murgatroyd,[6] however, injected citrated blood containing 720,000

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[1] Lane, C., *Lancet*, 1929, 1291.

[2] Lane, C., *Lancet*, 1933, 399.

[3] O'Connor, F. W., *Porto Rico J. Pub. Health and Trop. Med.*, 1931, **6**, 263.

[4] O'Connor, F. W., and Hulse, C. R., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1932, **25**, 445.

[5] Low, G. C., and Manson-Bahr, P. H., *Lancet*, 1933, 455.

[6] Murgatroyd, F., *Lancet*, 1933, 610.

microfilariae into a human volunteer 2 hours after withdrawing the donor's blood, but never recovered microfilariae from the recipient's blood. Rao[7] kept microfilariae alive *in vitro* at 4°C. under aseptic conditions for 4 to 6 weeks. He concludes from collateral evidence that the duration of the life of the embryos in the human body is about 70 days.

Dirofilaria immitis is an infection of the dog, the adult worms occurring chiefly in the right ventricle and pulmonary arteries and their microfilariae exhibiting a nocturnal periodicity, although not so marked as in *Wuchereria bancrofti*. Fülleborn[8] injected 6.25 cc. of blood, containing approximately 450,000 microfilariae, from an infected dog into a young puppy. These microfilariae persisted in the blood of the recipient for 9 months but showed no periodicity and were present in only about one-third of the expected number. He believed that the majority of the larvae withdrew to the lungs and only a fraction got into the circulation. Unfortunately the possibility of natural infection by mosquitoes or *via* the placenta of the mother was not entirely excluded.

The present writers transfused heavily parasitized blood at 5 P. M., March 29, 1934, into an uninfected 12-pound dog, shipped from Chicago (well outside the endemic area). The animal was kept in a doubly screened cage before and after the experiment. One hundred forty cc. of blood containing 27,475 microfilariae per cc. were transfused directly into the femoral vein, using the Gillentine-DeBakey syringe[9] after previous removal of an equal amount of blood. This latter blood was citrated and injected into the donor which weighed 10 pounds. Estimating one-twelfth of the body weight of the dog to be blood, the recipient had lost and later received approximately one-third of its blood. Therefore the number of microfilariae which might be expected to appear in its blood, if an even distribution resulted, would be about 9000 per cc. Actually only 8% of this number ever appeared in the peripheral bloodstream.

Counts of microfilariae were made upon the donor and recipient every 2 hours for a 24-hour period before and after the transfusion (Table I). Daily counts were then made at 5 P. M. for a week and subsequently, weekly counts (Table II). These figures are averages of counts on 2 samples of 8 cmm. each for the donor and of 20 cmm. each for the recipient.

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[7] Rao, S. S., *Indian Med. Gaz.*, 1933, **68**, 3; *Abst. in Trop. Dis. Bull.*, 1933, **30**, 705.

[8] Fülleborn, F., *Beihefte Arch. f. Schiffs-u. Tropenhyg.*, 1908, **12**, 6.

[9] Gillentine, W. H., and DeBakey, M. E., *New Orleans M. and S. J.*, 1933, **86**, 100.

TABLE I.
Transfusion.
No. microfilariae per cc.

Time	Donor		Recipient	
	Before	After	Before	After
5 P.M.	21,400	19,875	0	563
7 "	25,190	18,250		750
9 "	26,440	8,000		275
11 "	41,875	8,000		250
1 A.M.	44,625	9,063		375
3 "	47,000	9,425		175
5 "	31,500	8,875		300
7 "	16,325	6,000		350
9 "	17,625	13,750		375
11 "	17,750	9,350		425
1 P.M.	21,550	8,750		250
3 "	27,475	10,037		200

TABLE II.
No. microfilariae per cc.

Date—1934	Donor	Recipient
3/31— 5 P.M.	6,875	400
4/2 — 5 "	10,475	450
4/3 — 5 "	10,125	400
4/4 — 5 "	10,000	550
4/5 — 5 "	15,600	550
4/13— 5 "	21,650	750
—11 A.M.	35,875	75
4/19— 5 P.M.	38,750	100
—11 "	61,000	100
4/20— 5 A.M.	34,250	75
4/27— 5 P.M.	43,075	200
5/4 — 5 "	44,500	50

A study of Tables I and II indicates that the filarial count of the donor was markedly reduced by the removal of approximately one-third of the dog's blood, and remained lowered for 6 days, when it began to rise and had returned to almost the original level in 2 weeks (April 13). Counts done at 6-hour intervals on April 19-20 revealed that a greater number of microfilariae was present than before transfusion and that the usual periodicity reappeared. (Injection of sodium citrate was shown to produce no apparent reduction in numbers of microfilariae.) Daily production and destruction of the microfilariae would have returned the count to normal levels within 24 hours. Active microfilariae were found in the recipient's blood for 10 weeks, with only a slight variation during the first 2 weeks. The fact that the majority of embryos disappeared almost immediately after transfusion into the recipient may be due to their filtration by the viscera, particularly the lungs, as suggested by Fülleborn.[8]

It is apparent that, in the case of the dog heartworm-infection, periodicity cannot be explained on the basis of cyclical parturition and daily destruction of the larvae.

7433 P

Observations upon the Complement Titre in Experimental Leukopenia and Leucocytosis.*

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In connection with the causation of the Schultz syndrome or so-called agranulocytic angina, allergy has been considered as a factor.[1, 2] In observations upon allergy Deutsch and Weiss[3] have shown that the complement titre was markedly lowered and in anaphylactic shock, apparently absent.

During certain studies upon the production of granulocytopenic leucopenia with bacterial toxins[4, 5] we considered that it would be of interest to make observations upon the complement content of animals in which these cells had been depressed. As another factor in the production of granulocytopenic leucopenia we added the use of benzene inoculations. In addition to the animals wherein the leucocytes were depressed, others were employed in which experimental leucocytosis had been provoked.

In the present experiments 36 guinea pigs and 18 rabbits were employed. The complement titre of all rabbits used was found to be approximately 1/10 the strength of that of the guinea pigs. Seventeen guinea pigs in which granulocytopenic leukopenia was produced with *in vivo* prepared toxic filtrates of *B. enteritidis* and *B. typhosus* were bled from the heart. Eight rabbits were injected

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* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

[1] Pepper, O. H. P., *Calif. and Western Med.*, 1931, **35**, 173.

[2] Editorial, *J. Am. Med. Assn.*, 1933, **101**, 368.

[3] Deutsch, F., and Weiss, E., *Med. Klin.*, Berlin, 1933, **29**, 1402.

[4] Harris, W. H., and Schattenberg, H. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 843.

[5] Schattenberg, H. J., and Harris, W. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 847.

with benzene in olive oil over a period of 7 to 9 days with the production of leukopenia after which blood was withdrawn from the heart. The leukopenia could be more consistently produced in the guinea pig than in the rabbit.

In the production of experimental leucocytosis as previously found by us,[6] antigens of staphylococcus, milk, and *B. typhosus* injected intravenously in rabbits provoked the higher leucocytic responses of the group employed. In the present work, therefore, 15 guinea pigs and 5 rabbits were injected with sterile milk or suspensions of killed *Staphylococcus aureus*. The rabbits injected intravenously yielded a more constant and higher leucocytosis than the guinea pig.

In both the leucopenia and the leucocytosis the depression or increment of these cells were maintained for at least several days in order that the effect, if any, upon the complement may be more definite.

The sheep cell hemolytic series was employed in the titrations using 0.5 cc. of a 2% suspension of washed sheep cells. The hemolytic unit was first determined with the complement of control animals and the sera of the experimental animals paralleled with

TABLE I.

Complement Titration.

Animal	Count	Amount of Diluted Complement							
		.1 cc.	.2 cc.	.3 cc.	.4 cc.	.5 cc.	.6 cc.	.7 cc.	.8 cc.
Leucopenia—									
G.P. No. 14	3,400	++	+	±	—	—	—	—	—
G.P. No. 15	3,200	++	±	±	—	—	—	—	—
G.P. No. 17	3,000	+++	++	+	±	±	—	—	—
Rabbit No. 24	3,400	++	—	—	—	—	—	—	—
Rabbit No. 25	2,800	++++	+	±	—	—	—	—	—
Leucocytosis—									
G.P. No. 16	14,200	++++	++++	+++	++	±	±	—	—
G.P. No. 18	16,800	+++	++	±	±	—	—	—	—
G.P. No. 21	16,000	++++	+	±	—	—	—	—	—
Rabbit No. 26	27,200	++++	+++	+	—	—	—	—	—
Rabbit No. 27	41,600	++++	++	±	—	—	—	—	—
Controls—									
G.P. No. 1	9,800	+++	±	—	—	—	—	—	—
G.P. No. 2	8,200	++++	+++	±	—	—	—	—	—
G.P. No. 3	7,800	++++	+++	+	±	—	—	—	—
R. Bloods*	8,800	++++	++	±	—	—	—	—	—

++++ = No hemolysis.
+++ = ¼ "
++ = ½ "
+ = ¾ "
± = Almost complete hemolysis.
— = Complete hemolysis.

Guinea pig complement 1:20.
Rabbit complement 1:10.
* R. Bloods = average count and tested sera of 6 rabbits.
G.P. = Guinea pig.

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[6] Harris, W. H., and Schattenberg, H. J., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 265.

this unit as a guide. Because of the known variation of the normal complement curve, all series of animals including controls were bled and tested at the same time. The time of titration of the different series, after withdrawal of blood, varied from 4 to 18 hours.

Table I demonstrates the results obtained for representative animals of the different series and controls. In general it may be stated that no marked or clear cut difference in the complement content is manifested in animals showing leukopenia or leucocytosis as compared to the controls. Upon closer analysis there is an indication that leukopenic animals have a slightly higher complement titre than either the normal animals or those showing leucocytosis. In certain of the animals wherein benzene injections had been employed, the complement titre was high even just prior to death. The complement content of the sera of the animals with leucocytosis was quite similar to that of the controls.

While complement may be reduced or absent in allergy and this latter factor may play a rôle in the causation of the Schultz syndrome, our preliminary results do not show any reduction of the alexin of the sera of animals in which experimental leukopenia was produced.

7434 P

Barbiturate-Strychnine Antagonism in the Spinal Cat.

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Barbiturates are generally thought to act chiefly on the brain,[1] strychnine to exert its convulsant effects by an action on the cord.[2] If this were an absolute difference between the two, it would be difficult to see how the barbiturates could act as effective antagonists to strychnine, as numerous observers[3] have shown.

To test whether this antagonism does exist in the cord, the writers have made use of 9 spinal (decapitate) cats, prepared by the Sherrington method.[4] Strychnine sulphate was administered subcutaneously in doses of 0.75 or 1 mg. per kg. Violent convulsions followed in all cases, marked by typical repeated rigid extensor tetanus. When this stage was reached, a barbiturate solution was injected intravenously. This injection was followed in each case instantly by muscular relaxation. Threatened or actual recurrence of tetanus was met by further doses of barbiturate. The animals survived for varying lengths of time from 24 minutes up to several hours after tetanus appeared. Three control animals receiving strychnine alone showed recurrent tetanus for 20 to 80 minutes after its onset. All doses were based on weight of intact cat.

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[1] Sollman, T., Manual of Pharmacology, 4th Ed., 1932.

[2] Dusser de Barenne, J. G., *Physiol. Rev.*, 1933, **13**, 325.

[3] Zerfas, L. G., and McCallum, J. T. C., *Curr. Res. Anesth. and Analg.*, 1929, **8**, 349. Dawson, W. T., and Taft, C. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 917. Llopis Llorente, R., *Cron. Med. Valencia*, 1932, **36**, 58. Swanson, E. E., *J. Lab. and Clin. Med.*, 1932, **17**, 325. Haggard, H. W., and Greenberg,

The results are summarized in Tables I and II.

TABLE I.
Barbiturate-Strychnine-Antagonism in Spinal Cat.

Strych. Sulph. Cryst. mg./kg.	Min. to tetanus	Barbiturate, Initial dose in mg./kg.	Further tetani and procedure	Further doses Barbiturate X size of each in mg./kg.	Min. survival after onset of tetani
0.75	32	Na pentobarbital, 2%; 10	1 tetanus; 10 mg./kg. repeated	0	95+
0.75	40	Na amytal 10%; 10	0	0	68-81
1.00	39	Na phenobarbital, 10%; 40	0	1 x 40	43-48
1.00	42	Alurate, 10%; 33	0	2 x 16	92+
1.00	39	Dial, 10%; 10	0	11 x 5	69+
1.00	9	Pernocton, 1% (Na salt); 1	1 weak tetanus	0	24-37

TABLE II.
Strychnine Subcutaneously in Spinal Cat (Strych. Sulph. Cryst. 1.0 mg. per kg.).

Cat No.	1	2	3
Min. to onset of tetanus	5	14	19
Time tetani continued to occur	20	60	80
Time strong tetani continued to occur	12	38	55
No. of strong tetani	7	28	10
" " weak "	2	8	5
Min. survival after onset of tetanus	43-59	86-91	80-83

L. A., *J. Am. Med. Assn.*, 1932, **98**, 1133. Simon, I., *Arch. di farmacol. sper.*, 1932, **54**, 55. Barlow, O. W., *J. Am. Med. Assn.*, 1932, **98**, 1980. Wheelock, M. C., *J. Am. Med. Assn.*, 1932, **99**, 1862. Kempf, G. F., McCallum, J. T. C., and Zerfas, L. G., *J. Am. Med. Assn.*, 1933, **100**, 548. Swanson, E. E., *J. Lab. and Clin. Med.*, 1933, **18**, 933. Stalberg, S., and Davidson, H. S., *J. Am. Med. Assn.*, 1933, **101**, 102. Maloney, A. H., *J. Pharmacol. Exp. Therap.*, 1933, **49**, 133. Fenton, B. C., *J. Am. Med. Assn.*, 1933, **101**, 1333. Koumans, A. K. J., *Klin. Woch.*, 1934, **13**, 103.

[4] Liddell, E. G. T., and Sherrington, Sir Charles, Practical Mammalian Physiology, 1929.

Comment on Tables. "Survival"—disappearance of reflexes and of heart beat were usually nearly simultaneous, the survival period being between limits given. *Notes on individual experiments.* Na pentobarbital—recurrence of tetanus after 26 minutes shows that the strychnine dosage used is capable of causing a prolonged tetanic stage; observation was interrupted 70 minutes after the second tetanus, the preparation was left on artificial respiration and was in good condition 4½ hours later. Na amytal—it will be noted that the abolition of tetani may result from even very moderate dosage with barbiturate, but that this does not necessarily restore to the spinal cat preparation its normal viability of many hours. Alurate in diethylamine solution—92 minutes after onset of tetanus the preparation was in very good condition; observation terminated. Dial, in urethane-monoethyl urea solution, these bases present in amounts claimed not greatly to affect dial action[5]—the preparation was left on artificial respiration and found in good condition 2 hours later. Pernocton—a weak tetanus occurred 14 minutes after the first, and the reactivity then spontaneously and rapidly diminished to zero.

The present work is, of course, purely preliminary. It indicates, nevertheless, the existence of a barbiturate-strychnine antagonism in the cord animal. Experiments of a more quantitative nature are in progress.

7435 P

Electrocardiographic Changes in Acute and Chronic Pericarditis in the Goat.

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Clinical electrocardiographic experiences emphasized to us the similarity of the subjective, physical and electrocardiographic findings in hemopericardium and acute fulminating pericarditis, but we also noted electrocardiographic changes in the subacute or chronic healing stages of pericardial inflammation.[1] In order to get more exact information, Cohnheim's[2] classical experiments were repeated but instead of using oil or saline as Katz, Feil and Scott[3] and the Foulgers[4] had done in the dog, we used air, oxygen and blood as well as saline pressure in the pericardium of the goat. (Fig. 1.)

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[5] New and Non-official Remedies, 1934.

[1] Herrmann, G., and Schwab, E. H., *Trans. Assn. Am. Phys.*, 1934, **49**, in press.

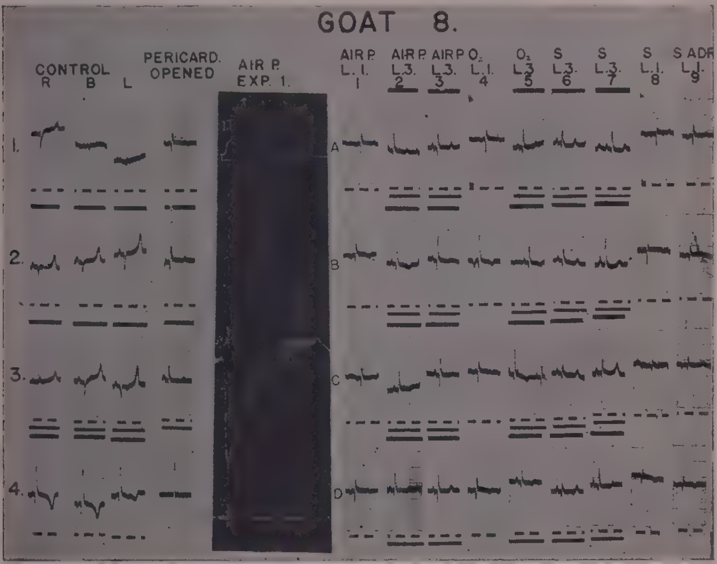

FIG. 1.

Electrocardiographic and kymographic tracings taken as control and during the acute air, oxygen and saline pressure experiments in Goat 8. The control electrocardiograms in leads 1, 2, 3, and 4 are shown first with the goat on his right side R, then on his back B, and then on his left side L; again after the chest and pericardium were opened and the pericardial cannula was in place. Under AIR P. EXP. I is shown the kymographic record of intrapericardial pressure, time during which the electrocardiogram was taken and the mean carotid blood pressure with as base lie a time marker in seconds. Then follow electrocardiograms taken at the beginning A; at the 10 mm. IP pressure height B; at the falling to 5 mm. IP pressure C; and at the return to the baseline of the IP pressure D. Exp. 1, 2, 3, with air pressure in leads 1, 3, and 3, Exp. 4 and 5, with oxygen in leads 1 and 3; Exp. 6, 7, and 8, with saline in leads 3, 3, and 1; and Exp. 9, saline in lead 1 with an injection of 0.1 cc. of adrenalin solution when the carotid pressure was at its lowest.

We also found, as others[3] have a direct relationship between intrapericardial pressure and electrocardiographic changes. After the acute pressure experiments (Fig. 1) protein solution or bacterial cultures were introduced into the sutured closed pericardium and the subsequent developments studied. (Figs. 2 and 3.)

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[2] Cohnheim, J., *The New Sydenham Society*, London, 1889, **1**, 21.

[3] Katz, L. N., Feil, H. S., and Scott, R. W., *Am. Heart J.*, 1929, **5**, 77.

[4] Foulger, M., and Foulger, J. H., *Am. Heart J.*, 1932, **7**, 744.

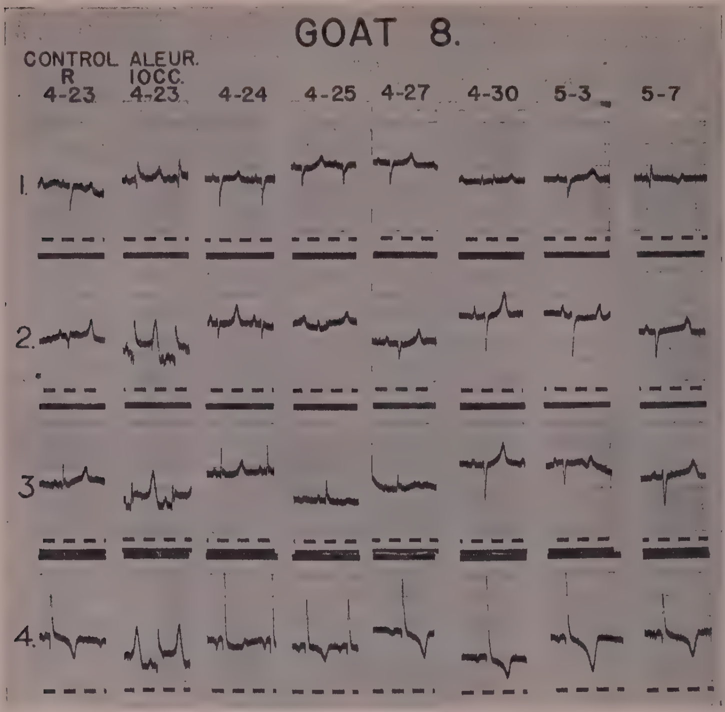

FIG. 2.

Goat 8, electrocardiographic tracings in leads 1, 2, 3, and 4 from above downward, taken as control and directly after filling the pericardium with saline and 10 cc. of protein solution and on several dates afterward, 4-24 to 5-7. It is to be noted that the acute positive elevations in all leads on 4-23 (10 cc.) disappear and subsequent T wave changes appear.

The elevation of the RST sector in all 4 leads was produced as shown in Fig. 2 (4-23) by the acute great increase in intrapericardial pressure, also lesser RST interval changes with slight elevation, upward convexity and negative T waves. (Fig. 1.) These changes were preceded by, and probably chiefly the result of, the sharp drop in peripheral BP that resulted from the auricular collapse incident to the increased intrapericardial pressure. (Fig. I KYMO. AIR. P. EXP. I.) The electrocardiographic changes disappeared shortly after the intrapericardial pressure was reduced or as the pericardium stretched or the fluid was absorbed. The electrocardiographic findings in hemopericardium or acute fulminating exudative pericarditis may be explained on the same premises.

As the chemical or bacterial pericarditis developed in a few days

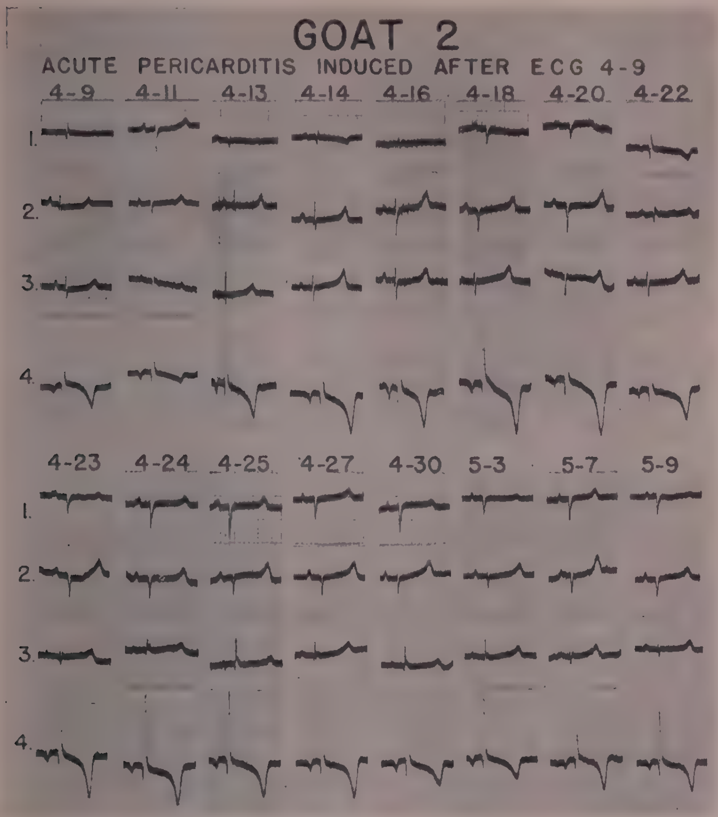

Fig. 3.

Goat 2, like the above except that only 3 cc. of the protein solution were injected. The more chronic cycles of T wave changes especially in lead 1 are to be noted on the various dates from 4-9 to 5-9.

there evolved ST interval changes and T wave negativity beginning in lead 1 and progressively developing in other leads and then slowly retrogressing as healing took place. (Figs. 2 and 3.) These findings as those in the human cases were accompanied by an epicarditis and a subepicardial myocardial involvement. The evolution and disappearance of the changes is more rapid in sterile chemical pericarditis. Recurrent phases of T wave changes may appear in the more subacute inflammatory processes. Thus the 2 types of electrocardiographic changes in pericarditis, the acute and the subacute are accounted for.

Minnesota Section.

University of Minnesota, May 16, 1934.

7436 C

Calcium and Coagulation of Blood.

F. H. SCOTT AND CAROLINE CHAMBERLAIN.

From the Department of Physiology, University of Minnesota.

Vines[1] called attention to the fact that it requires about 3 times the amount of oxalate to prevent the coagulation of blood than would be expected from the total amount of calcium in the blood. This was confirmed by Loucks and Scott.[2] Vines also called attention to the fact that on recalcifying coagulation occurred before all the oxalate was neutralized. Vines used small amounts of blood and used a biological method for calcium determination and his determinations of the amount of fibrin formed are only relative. It was felt that it was desirable to repeat some of the recalcifying work using larger amounts of plasma. The calcium determinations were made by the method of Kramer and Tisdall[3] while the fibrin formed, was washed, dried and weighed. Dogs that had been anesthetized with nembutal were the source of the blood used.

In a number of cases the amount of oxalate necessary to prevent coagulation was determined and then by recalcification of samples of plasma which had not clotted to see if they would clot at the same deficiency of calcium. Samples of plasma from 15 to 150 cc. were used in these experiments but for comparison all figures are given as if 100 cc. had been used. In all cases a sample of blood was permitted to clot and the calcium estimated in the serum. Loucks and Scott[2] showed the difference in calcium between plasma and serum was within limits of experimental error. To prevent evaporation while centrifuging the centrifuge tubes were always capped with a rubber membrane (Collins and Scott[4]). All the recalcifying experiments were done at room temperature.

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[1] Vines, H. W. C., *J. Physiol.*, 1921, **55**, 86.

[2] Loucks, Milo M., and Scott, F. H., *Am. J. Physiol.*, 1929, **91**, 27.

[3] Kramer, B., and Tisdall, F. J., *J. Biol. Chem.*, 1921, **47**, 415.

[4] Collins, Dean A., and Scott, F. H., *J. Biol. Chem.*, 1932, **97**, 189.

The experiments were carried out as follows: Varying amounts of a 17.4% potassium oxalate solution were put into 15 cc. centrifuge tubes and 10 cc. of the blood run into them, thoroughly mixed and then larger amounts of blood run into bottles containing enough oxalate to prevent coagulation. The larger samples were centrifuged immediately and the plasma removed. The smaller 15 cc. tubes were permitted to stand over night and then centrifuged and the calcium or oxalate estimated in the clear fluid. Typical results like the following were found:

Serum calcium = 10.1 mg. per 100 cc. All tubes of shed blood went solid until there was oxalate sufficient to make a deficiency of calcium of 32.8 mg. per 100 cc. Tubes with a calcium deficiency of 29.2 and 31.1 mg. per 100 cc. went solid. The larger amounts of plasma from the bottles was found to have a calcium deficiency of 38.2 mg. When less than 2 mg. of calcium were added (enough to neutralize only 1/20 of the oxalate present) there was a slight formation of fibrin. In order to get a solid clot one must add approximately enough calcium to neutralize about ⅓ to ¾ the calcium. In the above case the tubes did not go solid until the Ca deficiency had been reduced to 11.4 mg. In other cases it has gone solid with only ⅓ of the oxalate neutralized. The figures for decalcification and recalcification therefore do not indicate any fixed relationship.

As the above figures show one can obtain some fibrin on recalcification when there is still a great excess of oxalate present. One of the surprising things about the partial neutralization of the oxalate is that although there is oxalate present the influence of the calcium is felt for a long time as shown by experiments like this.

Plasma calcium deficiency = 30.1 mg. per 100 cc.
7.5 mg. Ca added after 2 hours slight clot.
" 4 " fair "
" 27 " enough clot so beaker could be

inverted. The control without calcium remained fluid. Likewise if one adds a small amount of calcium and lets stand for a time and then removes the fibrin one will get another crop of fibrin. For example a plasma with a calcium deficiency of 28.5 mg. had 14.4 mg. Ca added. After 6 hours it was solid and 0.428 gm. of fibrin were removed. On standing 14 hours longer 0.076 gm. more had formed. The calcium deficiency actually found at the end was 14.4 mg. per 100 cc.

As one adds more and more calcium more fibrin is formed. One obtains figures like the following:

Ca deficiency 38.2 mg. per 100 cc.		Ca deficiency 33.9 mg. per 100 cc.	
Ca added	Fibrin formed	Calcium added	Fibrin formed
1.9	0.024	8.3	.1584
3.8	.020	16.6	.3844
7.6	.053	24.9	.3456
11.4	.052	33.3	.3128
15.2	.049	49.9	.5664
19.1	.046	66.7	.6408
22.8	.064		
26.6	.092		
30.4	.358		
34.2	.580		
38.2	.603		
41.8	.716		

It is seen from the above that the relationships of calcium are by no means simple. That the conversion of fibrinogen into fibrin can take place in the absence of inorganic calcium is certain. When inorganic calcium is added to decalcified plasma the decalcified thrombin can compete at first against potassium oxalate for the possession of the calcium and active thrombin is formed. The active thrombin exerts its action for some time even in the presence of oxalate until it is again inactivated by the loss of calcium. Loucks and Scott[4] showed it takes some time for oxalate to destroy the activity of thrombin. (It is extremely difficult or impossible to destroy it by dialysis.) The regeneration of thrombin, when inorganic calcium is added, is however immediate.

Attention is called to the similarity of the calcium and kephalin in regard to thrombin. Mills[5] showed that thrombin passes into metathrombin because the kephalin goes over to the other blood proteins. Metathrombin may be converted to thrombin by the addition of kephalin and it will pass into metathrombin again as it loses it kephalin to the other proteins again.

7437 C

Glucose Tolerance.

ESTHER M. GREISHEIMER AND EDITH GOLDSWORTHY.

From the Department of Physiology, University of Minnesota.

Joslin[1] states that diabetes is hereditary in 25% of the cases. In view of this, the authors were interested in determining whether or not a tendency toward, or an early indication of, diabetes mellitus could be noted by some slight abnormality in the response to the glucose tolerance test in relatives of diabetics. A preliminary study[3] left the authors unsatisfied, so the work was continued for a time.

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[5] Mills, C. A., *Am. J. Physiol.*, 1926, **76**, 651.

[1] Joslin, E. P., *Diabetic Manual*, 1929, 4th edition, 145.

In conducting the test, each subject was asked to eat a balanced diet during the previous week, as the diet may markedly influence the results.[2] The conditions were not ideal, since the term "balanced diet" is interpreted in various ways; however, it was impossible to keep the subjects on a known diet before the test. A 12 to 14 hour fast preceded the test. The sugar was determined in the fasting blood, then one gram of glucose per kilogram of body weight was given in the form of lemonade. The blood sugar was determined again one-half hour, one hour, 2 and 3 hours after the ingestion of the glucose. The sugar was determined in duplicate by the Shaffer-Somogyi method.[4]

The subjects included 35 women and 18 men with maternal diabetic relatives, 20 women and 14 men with paternal diabetic relatives, and 8 women and 1 man with diabetic brothers or sisters. Of the entire group, diabetes mellitus had occurred in parents and grandparents in 9 women and 6 men; in 8 women and 1 man diabetes mellitus had occurred in both maternal and paternal relatives.

The results are presented in Table I; the glucose values are expressed as mg. %.

TABLE I.

	Fasting	½ hr.	1 hr.	2 hr.	3 hr.
Maternal diabetic relatives					
35 women	85	127	103	79	76
18 men	82	123	106	76	75
Average for above group	84	125	104	78	75
Paternal diabetic relatives					
20 women	81	124	97	79	73
14 men	81	130	108	76	68
Average for above group	81	126	102	77	71
Diabetic brothers or sisters					
8 women and 1 man	83	107	85	73	74
Diabetic parent and grandparent					
10 women and 5 men	89	131	124	82	80
Maternal and paternal diabetic relatives					
8 women and 1 man	82	133	100	73	73

It is evident that no striking abnormalities are present in the subjects studied. The differences between the group with maternal diabetic relatives and that with paternal diabetic relatives are unimportant.

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[2] Greisheimer, E. M., and Goldsworthy, E., *Am. J. Physiol.*, 1933, **105**, [illegible].

[3] Greisheimer, E. M., Goldsworthy, E., and Thomas, G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1426.

[4] Shaffer, P. A., and Somogyi, M., *J. B. C.*, 1933, **100**, 695.

The group with diabetic brothers or sisters shows a flat curve; 4 of these subjects were members of one family. Although no pertinent information could be had by questioning, it was suspected that this family tended to eat more carbohydrate food than the balanced diet permits. Obesity was present in these siblings.

The group with diabetic relatives in the past 2 generations showed a slight delay in the fall, as evidenced by the higher value in the one hour sample than in the other groups.

On the whole, no abnormalities appear constantly in the glucose tolerance in subjects who have maternal diabetic relatives, paternal diabetic relatives, diabetic relatives in the 2 preceding generations, or in those who have both maternal and paternal diabetic relatives.

The present study indicates that one cannot predict a diabetic tendency nor see an early indication of diabetes mellitus by constant abnormality in glucose tolerance in those whose relatives are known to have diabetes mellitus.

7438 C

Effect of Deuterium Oxide on Rat Sarcoma R-39.

CHARLES E. REA and SAMUEL YUSTER. (Introduced by O. H. Wangensteen.)

From the Department of Surgery and The Cancer Institute and the Department of Physical Chemistry, University of Minnesota.

The work of Lewis[1] on the inhibiting effect of deuterium oxide (heavy water) on the germination of tobacco seeds and the investigations of Barnes[2] showing the suppression of cell division in spirogyra by dilute solutions of deuterium oxide led us to study its effects on tumor tissue. Recently Woglom and Weber[3] reported that 0.40 to 0.42% deuterium oxide had no demonstrable effect on the growth of mouse sarcoma 180 or mouse carcinoma 63. Unaware of the above work we have been studying the effect of deuterium oxide on rat sarcoma R-39 for the past few months.

The deuterium oxide was obtained and analyzed by one of us (S. Y.) through the courtesy of Dr. Lloyd H. Reyerson of the Department of Chemistry. It was refluxed with acid dichromate, distilled, refluxed with potassium permanganate, and distilled again. Analysis by a density measurement showed the water to contain 0.11% deuterium oxide. The water was sterilized by autoclaving before use. No effect was noted in 4 normal rats, and 2 guinea pigs that received one cubic centimeter of this heavy water intraperitoneally every third day for 4 doses. This is in line with the work of Macht and Davis,[4] who found no detectable effect in mice which were injected with 0.05% deuterium oxide.

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[1] Lewis, G. N., *J. A. C. S.*, 1933, **55**, 3504.

[2] Barnes, T. C., *J. A. C. S.*, 1933, **55**, 4332.

[3] Woglom, W. H., and Weber, L. A., *J. A. M. A.*, 1934, **102**, 1289.

Rats with rat sarcoma R-39 were received from Dr. F. C. Wood of the Institute for Cancer Research, Columbia University, and transplants from these tumors were made into young stock rats weighing 60-90 gm. This tumor was selected as it is reported to take in 100% of cases; in this study, however, we obtained takes in only 86% of cases. The rats successfully inoculated with the tumor were divided into 3 groups; one group of 50 rats had one cubic centimeter of 0.11% deuterium oxide injected in and around the tumor every other day until 10 doses were given; the second group of 50 rats received one cubic centimeter of sterile water in and around the tumor for a similar period of time; a third group of 20 rats received no treatment. The diet consisted of Champion Mink food throughout the experiment.

The results obtained were similar to those recently reported by Woglom and Weber. No effect on either tumor or host was observed in most of the rats to which 0.11% deuterium oxide had been given. The size and course of 44 of the tumors in this group were practically the same as those that received sterile water. Over two-thirds of the tumors in groups one and 2 were badly ulcerated by the end of the experiment. However, six (12%) of the tumors injected with heavy water seemed to show a more rapid growth and were slightly larger than any of the controls. The interpretation of this finding is not clear. While it may be due to individual variations in the rats, the rather high incidence (12%) and the fact that such large or rapid growth was not obtained in any of the controls, seems to make this finding more significant. Recently Barnes and Larson[5] showed that dilute solutions of heavy water actually stimulated the growth of spirogyra.

Conclusions. 1. Heavy water in concentrations of 0.11% had no demonstrable effect on 4 normal rats. 2. No effect was observed in 44 of 50 rats inoculated with rat sarcoma R-39 to which the same concentration of heavy water was given. 3. However, 0.11% deuterium oxide seemed to have a stimulating effect on rat sarcoma R-39 in 6 of 50 rats so treated.

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[4] **Macht, D. I., and Davis, M. D., *J. A. C. S.*, 1934, 56, 246.**

[5] **Barnes, T. C., and Larson, E. J., *J. A. C. S.*, 1933, 55, 5059.**

7439 C

Comparative Efficacy of Substances Employed in Prevention of Intra-Peritoneal Adhesions.

CHARLES E. REA AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota

In this study, the following substances were compared with regard to their relative effectiveness in preventing peritoneal adhesions in rabbits: defibrinated ox blood, defibrinated rabbit blood, amniotic fluid, amfetin,* sodium ricinoleate (1%), blood serum of dog, ether, hypertonic glucose (10%), normal saline, papain 1-50,000, air, air and sodium ricinoleate (1%), air and amfetin.

The method was as follows: under ether anesthesia, the abdomen of the rabbit was opened and a culture taken with sterile precautions. Intraperitoneal adhesions were produced by roughening the peritoneum and intestines with a knife or rubbing with gauze, and painting the raw surfaces with tincture of iodine. In 12 animals so treated (controls) 2 died from the procedure, and of the 10 surviving 9 developed peritoneal adhesions (90%). Twenty cubic centimeters of the substance to be tested were introduced into the peritoneal cavity after the iodine was applied. When air was also added, 20 cc. of the solution and 60 cc. of the air were used. About 10 rabbits were used in each series. In the 4 best series the effectiveness was studied in another 10 rabbits to make the number of animals used (20) significant. The rabbits were allowed to live one week before being killed, when a culture was again taken. The peritoneal reaction was studied. Adhesions were graded as slight, medium or dense. The results of the experiments are tabulated in Table I.

Results. The 4 substances that were most effective in preventing adhesions in this study were: Sodium ricinoleate (1%), 85%; Papain 1-50,000, 75%; Amfetin, 70%; defibrinated rabbit's blood 65%.

Ochsner and Garside demonstrated that papain and trypsin are inactive in the presence of infection. Sodium ricinoleate, or castor oil soap, not only prevented adhesions most often, but has the added

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* Lilly trade name for amniotic fluid.

TABLE I.

Treatment	No. of rabbits	Procedure	Adhesions—No. of cases — Time—1 wk. — Dense	Medium	Slight	None	Operative mortality	Remarks
Controls	12		5	3	1	1	2	
Sodium ricinoleate 1%	10 (20)		0 (0)	0 (0)	0 (1)	9 (17)	1 (2)	Numerals in parenthesis refer to total number of cases, using 20 animals
Papain 1·50,000	10 (20)		0 (0)	0 (0)	1 (2)	8 (15)	1 (3)	
Amfetin	18 (20)		0 (0)	1 (1)	1 (2)	6 (14)	0 (2)	
Defibrinated rabbit blood	10 (20)		0 (0)	1 (1)	2 (3)	6 (13)	1 (2)	
Defibrinated ox blood	10	Roughening peritoneum + tincture of iodine + 20 cc. solution to be tested. When air was also added, 20 cc. solution + 60 cc. air were used.	0	1	1	6	2	
Amniotic fluid (dog)	12		0	0	4	6	2	
Air	10		0	1^{a}	1^{b}	2^{c} 3^{d}	3	
Air + amfetin	10		0	1	1	5	3	
,, + sodium ricinoleate 1%	10		0	0	1	7	2	
Blood serum (dog)	10		0	0	3	5	2	
Ether	9		0	1	4	4	0	10 cc. ether used
Hypertonic glucose	10		0	0	2	5	3	
Normal saline	10		0	1	4	3	2	

[a] 4 days. [b] 3 days. [c] 4 days. [d] 5 days.

advantage of acting in either in a sterile or infected peritoneum. Its action on bacteria is a surface tension phenomenon; it destroys the cell membrane of the bacteria (Larson[1]). While *B. coli* and *Cl. welchi* will grow in sodium ricinoleate, papain, amfetin, and defibrinated blood, they are rendered nonpathogenic by sodium ricinoleate. Its action in preventing adhesions, except for its effect in limiting bacterial growth, is not clear. The manner in which it works is more than by just mechanical separation of the bowel, for in rabbits most of it is absorbed within one hour (B. Olson[2]). Studies on the peritoneal fluid of rabbits using supravital staining methods (neutral red) would suggest that half the histiocytes are either killed or injured by the sodium ricinoleate, judging from the amount of dye in these cells. Thus, sodium ricinoleate may destroy the cell membrane of the histiocytes as well as bacteria, thereby limiting the repair process. It emulsifies fibrin *in vitro;* papain actually digest fibrin, while amfetin and defibrinated rabbit's blood have no effect.

Cultures were taken from the peritoneal cavity before and after the adhesions were produced. It is interesting that some type of bacterial growth was cultured in approximately half (42 out of 79) of the peritonea of apparently normal rabbits before the trauma and iodine were applied. The technique was checked by members of the Surgery and Bacteriology Departments and no errors could be detected. Controls were taken routinely from the applicators and media. For the most part the growths were mixed cultures of Gram positive and negative rods and cocci; *B. coli* was never found. At death all cultures of the peritoneal cavity showed some growth; 20% of the cultures showed *B. coli* and 15% *Cl. welchi*. However, in 3 of the 9 cultures taken from rabbits that had received sodium ricinoleate, there was no demonstrable growth, and one of these had shown a positive culture of mixed organisms before operation.

It is the practice of this hospital when sodium ricinoleate is used to put 60 cc. in the peritoneal cavity. Our clinical results with it or any of the other substances are too meager to draw conclusions. However, since in this study, sodium ricinoleate was most effective in preventing peritoneal adhesions in rabbits, its further clinical use would seem warranted.

The addition of air to sodium ricinoleate (1%) or amfetin in these experiments did not appear to enhance the protecting mechanism against adhesion formation. In a few patients who have been operated upon repeatedly for intestinal obstruction of adhesive origin, air has been put into the peritoneal cavity, after a complete lysis or freeing up of the adhesions was done, to mechanically separate the gut from the abdominal wall during the healing phase. Surprisingly good results have been obtained in a few such instances.[3]

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[1] Larson, W. P., personal communication.

[2] Olson, B. J., personal communication.

Summary. Sodium ricinoleate, 1%, papain 1-50,000, and amfetin and defibrinated rabbit's blood were found most efficacious in obviating adhesion formation after painting the traumatized peritoneal surface with iodine. Sodium ricinoleate would appear to be particularly valuable in that it is not rendered inactive in the presence of infection.

7440 C

Character of the Gaseous Distension in Mechanical Obstruction of the Small Intestine.

JAMES S. HIBBARD AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

McIver[1] and his associates have shown that gaseous distension of the stomach after abdominal operations is largely due to swallowed air. In mechanical obstructions of the gut, McIver[2] believes that decomposition of intestinal contents is probably the most important source of the gaseous distension. Wangensteen and Rea[3] by excluding the swallowing of air by transecting the esophagus in the dog have indicated that swallowed air is probably the chief source of gaseous distension in mechanical obstructions of the small intestine as well.

In this study the nature of the gases present in the distended intestine was subjected to chemical analysis. Determinations were made in a group of dogs with terminal ileal obstructions, the gut being divided a few centimeters proximal to the ileo-cecal sphincter; in a similar group of dogs in which a preliminary esophagostomy had been made, and in a third group with closed intestinal loops.

The gases of the obstructed intestine were analyzed with the employment of the U. S. Bureau of Mines apparatus.* The gas sample was drawn over mercury into specially made gas tubes while the animals were under ether. In some analyses, brine was used in the gas apparatus instead of mercury, and this accounts for the lack of the estimation of the volatile basic group in some of the experiments.

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[3] Wangensteen, Owen H., *Arch. Surg.*, 1933, **26**, 933.

[1] McIver, M. A., Benedict, E. B., and Cline, J. W., *Arch. Surg.*, 1926, **13**, 588.

[2] McIver, M. A., *Am. J. Surg.*, 1933, **20**, 509.

[3] Wangensteen, O. H., and Rea, C. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1060.

TABLE I.
(Group A) Simple Ileal Obstruction.

Wt.	Duration	Fluid Content	Gaseous Content							Volatile Basic Group
lbs.	days	cc.	Amount cc.	CO_2 %	O_2 %	N_2 %	CH_4 %	H_2 %	H_2S %	%
55	3	150	100	—	—	—	—	—	—	—
50	2	—	—	4.70	10.92	61.+	0	0	4.68	+
42	3	300	150	6.20	1.48	71.+	0	0	7.86	+
48	3	290	200	12.71	1.11	60.+	0	0	14.00	+
42	3	150	220	12.19	0.69	70.+	0	0	1.99	+
46	3	220	290	9.36	1.00	74.+	0	0	0.92	+
44	3	235	320	9.10	0.52	70.+	0	0	4.33	+
55	3	1198	325	13.10	1.82	73.64	0	0	9.41	2.03
36	5	400	200	11.50	0.97	79.4	0	0	6.58	1.54
34	7	200	300	8.80	2.14	66.+	0	0	13.23	8.75
48	3	210	225	24.20	0.30	53.04	0	0.35	17.90	4.21
32	6	750	250	—	—	—	—	—	—	—
43	3	420	430	15.09	1.15	23.+	0	0.49	45.14	+
	Dead									
Average		373	234.5							

TABLE II.
(Group B) Simple Ileal Obstruction with Esophagostomy.

Wt.	Duration	Fluid Content	Gaseous Content							Volatile Basic Group
lbs.	days	cc.	Amount cc.	CO_2 %	O_2 %	N_2 %	CH_4 %	H_2 %	H_2S %	%
42	3	250	110	12.8	1.1	76.3	0	0	9.8	—
36	3	240	140	9.0	3.2	85.0	0	0	3.1	—
40	3	300	40	9.5	0.4	73.4	—	8.2	8.0	0.5
52*	3	250	100	12.	5.7	76.2	0	0	6.1	—
46*	3	225	160	—	—	—	—	—	—	—
39*	3	150	60	12.8	0.9	71.6	.3	4.	10.4	—
Average		236	100							

*Were fed a mixed diet previous to operation.

The composition of the gas appearing in all groups (Tables I, II, and III) was different in some respects than the composition occurring in other conditions reported in the literature. Nitrogen tended to come into equilibrium with the blood and surrounding tissue, decreasing to about four-fifths of an atmosphere. In the early hours of obstruction the oxygen content was found to be

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*Bulletin 197, U. S. Bureau of Mines, 41.

TABLE III.
(Group C) Closed Loop Obstruction of Small Intestine.

Wt.	Duration	Fluid Content	Gaseous Content							Volatile Basic Group
			Amount	CO_2	O_2	N_2	CH_4	H_2	H_2S	
lbs.	days	cc.	cc.	%	%	%	%	%	%	%
36	2	60	90	—	—	—	—	—	—	—
42	2	150	70	4.5	12.35	87.00	—	—	—	—
40	3	225	83	8.26	2.39	74.84	0	0	12.78	1.62
42	3	1050	40	10.00	4.00	—	—	—	3.00	Trace
30	5	130	10	—	—	—	—	—	—	—
44	8	200	100	—	—	—	—	—	—	—
38	3 Dead	600	40	5.12	2.8	51.+	0	0	15.36	+
46	3 Dead	—	—	18.40	1.02	11.94	0.13	3.25	62.74	+
52	3 Dead	—	—	13.17	1.75	37.34	0	2.33	42.20	3.21
26	4 Dead	350	20	—	—	—	—	—	—	—
Average		136	65.5							

quite high (10 to 12%), but after the 72 hour period its value fell to a uniformly low figure. Even in early obstruction, the carbon dioxide showed a decided approach to that found in the blood. Apparently this was due to its high diffusion rate. The average percentage of these gases agrees quite favorably with other conditions previously reported.

Hydrogen sulphide was present in all experiments and increased as the severity of the obstruction increased with a marked rise after death. The volatile basic group (ammonia, methyl amine, etc.) although not high, appeared quite consistently throughout and showed some increase in the later stages. Since the formation of this group of gases is entirely dependent upon protein putrefaction, the high percentage found as death approached suggests the marked degree of putrefaction present in the terminal stages of obstruction.

The results obtained in the combustible group of gases were entirely unexpected. Methane was present in only 2 experiments, and then only in a very small amount (0.13 and 0.30%). Similarly, hydrogen was consistently absent, occurring in only 2 animals (8.2 and 4.0%). The results obtained in the combustible group of gases can be explained on the basis of Kantor's[4] suggestion, *viz.*, that the greatest amount of combustible gas is formed in the colon by a specific bacterial action on cellulose. This explanation is supported by McIver, who found large quantities of combustible gases in paralytic ileus in which the samples were obtained by rectum.

Comparing the results in Table I with those in Tables II, and III, it is quite evident that the quantity of gas occurring in the closed loops is much less. The average amount found in the closed loops was 76 cc. as compared to 234.5 cc. occurring in open loops of a comparable length. Since the quantity of gas produced in the closed loops arose either from food decomposition or from the blood stream, since nitrogen gas can not originate from food material, it is obvious that the larger part of the gas present must have come from diffusion from the blood stream. Certainly, a much larger quantity than 30% of the gas found in the closed loops is formed by food decomposition, but through rapid absorption and nitrogen displacement it disappeared quite readily. The results of previous studies in this Department support these findings.[5]

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[4] Kantor, J. L., *Am. J. Med. Sci.*, 1918, **155**, 829.

Additional analyses are being made on clinical cases, and experimental large bowel obstruction, which will be reported at some subsequent time.

Summary. The average percentage composition of the different gases is as follows: (a) Nitrogen 70%; (b) carbon dioxide, 6-12%, approached that found in blood gases; (c) oxygen, 10-12%, with a decided drop after the 72 hour period; (d) combustible gases (methane and hydrogen), a very low percentage in only a few experiments; (e) hydrogen sulphide, 1-14%, with a marked increase after death; (f) volatile basic group (ammonia, methyl amine, etc.) 0.5-4.0%, with an increase with longer duration of the obstruction.

In a quantitative determination of the origin of gases occurring in small bowel obstruction, about 68% of the gas was estimated to have arisen from swallowed air. The amount formed within the body was 32%, of this about 70% originated from diffusion from the blood into the bowel lumen, and the remaining 30% arose from decomposition of food material.

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[5] Scott, H. G., Dvorak, H. J., Borman, C. N., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 902.

7441 C

Sexual Differences in Fasting Blood Sugar.

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Investigators have disagreed as to the changes in blood sugar during fasting; those who have reported a decrease have failed to note a sexual difference. Allen believed that the blood sugar is maintained at 100 mg.% through prolonged starvation, almost up to death. Joslin[2] found the blood sugar decreased from 120 to 90 mg.% during 4 days, in which a healthy woman fasted, save for small quantities of weak bouillon.

Weeks and his co-workers[3] observed 64 epileptics, who fasted 3 weeks. Sugar determinations were made only once a week; the sugar was reported as being maintained at an approximately even level. Acidosis was present, but not severe. Shaw and Moriarty[4] observed epileptic children during 10 to 14 day fasts. They found a steady diminution in blood sugar, with the minimum (46 mg.%) between 3 and 8 days, after which the sugar increased. They considered the diminution in blood sugar peculiar to childhood, since it was supposed that the blood sugar is not much diminished in adults during fasting.

Lennox and his co-workers[5] observed 16 men and 8 women during 3 to 21 day fasts. They noted that the blood sugar fell during the first week of fasting. Their minimum was 65 mg.%. Shope[6] studied one woman who fasted 6 days. He found that the blood sugar decreased to a minimum of 37 mg.%. As the fast continued, a secondary rise in blood sugar occurred. Deuel and Gulick[7] observed 5 men and 5 women during 7 day fasts. The women subjects showed ketosis before the men. It developed more rapidly and became more severe, as shown by both the urine analyses and the carbon dioxide combining power of the plasma. No blood sugar studies were made.

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[2] Joslin, E. P., *Carnegie Inst. of Wash. Pub.*, No. 323, 1923, 137.

[3] Weeks, D. F., Renner, D. S., Allen, F. M., and Wishart, M. B., *J. Metab. Res.*, 1923, **3**, 317.

[4] Shaw, E. B., and Moriarty, M., *Am. J. Dis. Child.*, 1924, **28**, 553.

[5] Lennox, W. G., O'Connor, M., and Bellinger, M., *Arch. Int. Med.*, 1926, **38**, 553.

[6] Shope, R. E., *J. Biol. Chem.*, 1927, **75**, 101.

[7] Deuel, H. J., Jr., and Gulick, M., *J. Biol. Chem.*, 1932, **96**, 25.

In spite of contradictory findings, several text-books hold to the view that there is no marked decrease in blood sugar during fasting. Macleod[8a] states that early in starvation the blood sugar becomes much lower than normal, after which it remains steady, but in the same text[8b], he contradicts this, and states that in complete starvation the blood sugar does not fall very much below the normal level. Starling[9] states that even during prolonged starvation the blood sugar does not fall notably below the amount present after a fast of only a few hours. Howell[10] states that even in prolonged starvation the sugar contents of the blood are kept at a normal level.

In view of the differences of opinion, it seemed desirable to make additional studies. The decrease in blood sugar during fasts of 60 hours has been determined in a group of 54 adults (28 women and 26 men), who were either students or instructors. Water, black coffee, and clear tea were allowed. Each subject did his usual work during the fast.

The blood was drawn by venepuncture after 12, 36, and 60 hours of fasting. The sugar was determined (in duplicate) by the Shaffer-Somogyi method. The following values were found; they are expressed as milligrams percent:

hr.		28 women	26 men
12	Min.	71	73
	Max.	95	95
	Aver.	83±0.70	85±0.45
36	Min.	56	64
	Max.	79	89
	Aver.	69±0.84	78±0.79
60	Min.	48	59
	Max.	80	92
	Aver.	62±1.05	67±0.82

The following differences between the sexes were found; the men had the higher values in every case.

12 hours	36 hours	60 hours
1.5±0.83	8.9±1.15	5.3±1.33

The total fall for women was 25.6%; of this, 67% occurred between 12 and 36 hours. The total fall for men was 20.6%; of this, 39.1% occurred between 12 and 36 hours. It is evident that the fall is more rapid in women during the early stages of the fast. It may be that women have less glycogen stored in the liver than men.

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[8] Macleod, J. J. R., Physiology and Biochemistry in Modern Medicine, 1926, 5th ed., (a) 792; (b) 875.

[9] Starling, E. H., Human Physiology, 1933, 6th ed., 666.

[10] Howell, W., Text-book of Physiology, 1933, 12th ed., 983.

It has been found that female rats have lower fasting liver glycogen values than male rats[11].

Qualitative tests for acetonuria were done at 36 and 60 hours. Twelve of the women showed acetonuria at the end of 36 hours, and 27 at the end of 60 hours. Six men showed acetonuria at the end of 36 hours, and all showed it at the end of 60 hours. These results agree with those of Deuel[7].

The average weight loss for women was 5.1 pounds; for men, 6.4 pounds. The range of weight loss for women was 3 to 10 pounds; for men it was 3⅞ to 12½ pounds. The women lost 1.8% of the body weight between 12 and 36 hours; the total loss was 3.8% of the body weight. The men lost 1.9% between 12 and 36 hours; the total loss was 3.6%.

In view of Haggard's findings[12], the subjects were asked not to smoke during the half hour preceding the taking of the blood each morning. Among the women fasters, there were 14 smokers and 14 non-smokers. Among the men there were 20 smokers and 6 non-smokers. The differences between the smokers and non-smokers were not consistent. The women smokers have lower values than the non-smokers; the differences were 7. mg. % at the end of 12 hours, 4. at 36 hours, and 9. at 60 hours. The men smokers had 3. mg.% more sugar than the non-smokers at 12 hours; at 36 hours the non-smokers were 3 mg.% above the smokers; at 60 hours the non-smokers were less than 1 mg. higher than the smokers. Smoking during the days of fasting, except during the half-hour preceding the venepuncture each day, has no consistent effect on blood sugar.

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[11] Greisheimer, E. M., *J. Nutrition*, 1931, **4**, 411.

[12] Haggard, H. W., and Greenberg, L. A., *Science*, 1934, **79**, 165.

7442 C

Comparison of Jenner-Kay and Bodansky Methods for Determining Phosphatase in Plasma and Serum.*

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Since the autumn of 1932 we have made phosphatase determinations in several hundred samples of plasma from cows' blood by the clinical method of Jenner and Kay[1]. This method as well as that of Kay[2], of which it is a modification, has been criticized by Bodansky[3, 4], because of unnecessarily large experimental errors; the method developed by Bodansky[3, 4] is more rapid and is believed by him to eliminate the errors inherent in the Kay and Jenner-Kay technics.

Before we began to employ the Jenner-Kay method in the routine analysis of bovine plasma we had satisfied ourselves that sufficiently uniform results could be secured when it was applied to the type of plasma samples employed by us for other plasma analyses, namely, total calcium and inorganic phosphorus. These samples are a composite of equal parts of plasma centrifuged at once from citrated blood obtained on 3 successive days. Preliminary data obtained by the Jenner-Kay method from normal heifers whose plasma showed widely different levels of phosphatase showed for example that samples containing 31.46, 36.16 and 36.92 units per 100 cc. on successive days showed 34.70 units in the 3-day composite against a calculated value of 34.85 units. The same composite sample analyzed 38.72 units phosphatase after 6 days' storage. Another animal with plasma phosphate values of 16.50, 16.28 and 16.76 units per 100 cc. on successive days showed 16.70 units for the composite against 16.51 units calculated from the individual samples. A third animal with plasma phosphatase of 6.18, 6.98 and 6.96 units per 100 cc. on successive days showed 7.84 units in the composite against a calculated value of 6.71. These data, which were subsequently verified by a very large amount of data which will not be reported at this time, suggest that the phosphatase content of the blood plasma in dairy cattle is to a considerable extent an individual characteristic. Furthermore, it seemed apparent that the Jenner-Kay technic is applicable to the determination of plasma phosphatase in composite samples within reasonable limits of accuracy. The results also suggest that greater errors may be expected when the phosphatase content is low and that some inaccuracy may result if the composite sample is not analyzed promptly.

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*Published with the approval of the Director as Paper 1258 Journal Series, Minnesota Agricultural Experiment Station.

[1] Jenner, H. D., and Kay, H. D., *Brit. J. Exp. Path.*, 1932, **13**, 22.

[2] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235.

[3] Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1931, **7**, 760.

[4] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

After the publication by Bodansky[4] of his study of errors involved in a phosphatase estimation we became interested in a comparison of his and the Jenner-Kay technic to samples of plasma and particularly to definitely proportional mixtures of plasma samples having widely different concentrations of phosphatase. The latter type of study has long been regarded as a satisfactory test of the reliability of an analytical procedure.

In a first comparison of the methods plasma from citrated and oxalated blood as well as the serum from 2 normal heifers, one with high and the other with low blood phosphatase content, as shown by previous analyses were analyzed simultaneously by the Jenner-Kay and Bodansky technics when freshly drawn and after 2 and 4 days. Bodansky[4] prefers serum to oxalated plasma, but has not reported data on citrated plasma which we have employed throughout; Kay[2] and Jenner-Kay[1] use oxalated plasma. For a number of years we have used the Fiske-Subbarow[5] method for inorganic phosphate and therefore employed it for the Jenner-Kay phosphatase determination. We found that the Fiske-Subbarow reagents give a precipitate with the Bodansky phosphatase substrate, making it necessary to employ the Kuttner-Cohen[6] method for inorganic phosphate with the Bodansky phosphatase method, which Bodansky recommends. Therefore the comparison of the methods for phosphatase likewise included a comparison of the results of phosphate by the Fiske-Subbarow and Kuttner-Cohen methods. All analyses were made in triplicate. Incubation was for 3 hours at 38° in the Jenner-Kay method and for one hour at 37°C. in the Bodansky method. Preserved samples were kept in the refrigerator.

It was found that the initial inorganic phosphate in the samples as determined by the Kuttner-Cohen method showed greater irregularity than by the Fiske-Subbarow method when the fresh, 2-day and 4-day old samples were compared. The average variations were 0.56 mg. % in the former and 0.26 mg. % in the latter. This may have been due in part to a lack of previous experience with the former method. Oxalated blood plasma sometimes showed higher inorganic phosphorus than citrated plasma by both methods, amounting on the average to 1.25 mg. %. No explanation suggests itself for this inasmuch as the samples represented the same bleeding. Regarding the enzyme units there was no greater uniformity among comparable samples from the same animal by the Bodansky method than by the Jenner-Kay method. Some uniformity was found in the ratio of the results by the two methods for the same animal but the ratio was clearly different for different animals, the J.K. units ranging from 1.8 to 3.3 times the Bodansky units in the cases studied. An increase in phosphatase activity was not noted in preserved samples.

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[5] **Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, 66, 375.**

[6] **Kuttner, T., and Cohen, H. R., *J. Biol. Chem.*, 1927, 75, 517.**

For the chief method selected for a comparison of the 2 phosphatase procedures 4 pairs of animals were chosen having in 3 instances widely different blood phosphatase concentration and in the fourth instance nearly the same phosphatase concentration. The individual samples of each pair were analyzed and also mixtures of these pairs in ratios of 1:2 and 2:1. All analyses were made on freshly prepared citrated plasma. The Fiske-Subbarow method for inorganic phosphate was used for the Jenner-Kay procedure and the

TABLE I.

Comparison of Jenner-Kay and Bodansky Phosphatase Units in Mixtures of Plasma of Determined Phosphatase Content.

		Phosphatase units per 100 cc. Jenner-Kay		Phosphatase units per 100 cc. Bodansky		Ratio $\frac{\text{J-K}}{\text{B}}$ units
Date	Sample	Found	Calculated	Found	Calculated	
11-6-33	E150	49.25		23.76		2.07
	2E150:1E78	36.25	33.78	16.05	16.20	2.26
	1E150:2E78	17.24	18.30	8.98	8.64	1.92
	E78	2.83		1.07		2.65
1-18-34	E150	48.00		24.53		1.96
	2E150:1E78	28.39	32.92	17.40	16.65	1.63
	1E150:2E78	19.75	17.83	10.22	8.77	1.93
	E78	2.75		0.89		3.09
1-22-34	E193	13.81		7.03		1.96
	2E193:1E191	15.48	15.04	8.19	8.01	1.89
	1E193:2E191	16.38	16.25	9.00	8.98	1.82
	E191	17.48		9.96		1.75
1-24-34	E150	40.59		27.20		1.49
	2E150:1E151	28.83	27.93	17.56	18.60	1.64
	1E151:2E150	15.92	15.35	10.00	10.01	1.59
	E151	2.72		1.41		1.93
1-26-34	E184	12.70		5.62		2.26
	2E184:1E183	8.91	9.01	3.87	3.81	2.30
	1E184:2E183	5.24	5.30	2.16	2.00	2.43
	E183	1.64		0.40		4.10

Kuttner-Cohen method for this analysis in the Bodansky procedure. The incubation procedures were according to directions. All analyses were made in triplicate.

Table I shows the results of these comparisons, the inorganic phosphate determinations being omitted for lack of space. It was evident, however, that the inorganic phosphate was estimated with essentially equal accuracy by the 2 methods, as judged by a comparison of the calculated values with those determined in the various mixtures of plasma; the mean percentage error was 0.75% for the Fiske-Subbarow method and 1.23% for the Kuttner-Cohen method. The results of this study also fail to show any superiority of the Bodansky method over the Jenner-Kay method for plasma phosphatase so far as accuracy of calculated vs. determined values is concerned. The range of errors for the Bodansky method was from 0.10 to 16.53% with a mean error of 4.36%, while the range of errors for the Jenner-Kay method was 0.80 to 13.76%, with a mean of 5.04%. The choice of method seems to be one of personal preference rather than significantly greater accuracy when applied in routine analysis. When only a few samples are to be analyzed the shorter incubation period of the Bodansky method is an advantage. For a larger series the longer incubation period of the Jenner-Kay method is definitely advantageous in routine work. Inasmuch as both methods are empirical and, in our judgment, equally accurate, it is unfortunate that there does not seem to be a more uniform ratio between the units arrived at by the methods. While the ratio of Jenner-Kay to Bodansky units is roughly 2:1 in our series, the range for individual samples is from 1.49:1 to 4.10:1.

7443 C

Fading of Methylene Blue-Acetone Solutions by Ultra-Violet Light.

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Recently Nurnberger and Arnow[1] found that solutions of methylene blue in water irradiated under a mercury-arc therapy lamp had their absorption spectra changed quantitatively when the rays from the lamp were filtered through quartz. If, however, the rays shorter than 270 millimicrons were absorbed by a suitable filter, then practically no change in the absorption spectrum, either in the visible or ultra-violet, occurred for exposures 2 hours in length. It was, therefore, concluded that the fading of methylene blue in water is not a satisfactory indication of ultra-violet ray intensity in general.

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[1] Nurnberger, Carl E., and Arnow, L. Earle, *J. Phys. Chem.*, 1934, **38**, 71.

Leonard Hill[2] and Webster, Hill and Eidinow[3] used the fading of methylene blue in acetone solutions when exposed to ultra-violet light to measure the intensity of the "physiologically active" part of the spectrum of sun light. Hill found his solutions to be most sensitive to wave lengths shorter than 320 mμ. In this respect, methylene blue in acetone and methylene blue in water behave alike, though it seemed indicated that the sensitivity of the former solution extended to longer rays.

Accurate intensity measurements require knowledge about the response of the indicator to the different wave lengths of the light used. The usual and essential procedure in the use of bolometers, thermopiles, photoelectric cells and other instruments used to measure intensity of radiation is to determine beforehand their sensitivity to different spectral regions. The same care, however, is not commonly observed when colored solutions are used, and therefore, the results are only approximate and are unsatisfactory for ultra-violet therapy. Colored solution indicators with known spectral sensitivity have, however, some advantages over the instruments mentioned. Particularly, the simplicity of the experimental apparatus gives to the method a more extensive usage than can be had with more costly equipment.

A convenient method for the determination of the color fading results from the characteristic absorption of visible rays. Methylene blue in aqueous solutions has absorption bands with a maximum at 670 mμ.[4] A measure of the amount of light absorbed at this peak can be used to calculate the quantity of methylene blue changed by the ultra-violet irradiation. These measurements are commonly made by means of a spectrometer in combination with some type of photometer. We used a Bausch and Lomb constant-diviation spectrometer and a polarization photometer of the Konig-Martin type.

The irradiated solutions were made from a 0.1 mg. per cc. standard solution of Merck's medicinal methylene blue in distilled water. Solutions of 3 different concentrations were used. Two of them consisted of 5.8 cc. and 58.0 cc. respectively, of the standard plus

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[2] Hill, Leonard, *Strahlentherapie*, 1929, **34**, 117.

[3] Webster, A., Hill, Leonard, and Eidinow, A., *Lancet*, 1924, **206**, 745.

[4] Stenstrom, W., and Lohmann, A., *Radiology*, 1931, **16**, 322.

30 cc. of acetone added to distilled water to make 100 cc. of solution. The third consisted of 5.8 cc. of the standard added to distilled water to make 100 cc. of solution. The solution which contained 58.0 cc. of the standard had nearly the same concentration of methylene blue and of acetone as Hill's solutions (loc. cit.). The solutions were kept in the dark all of the time except when samples for irradiation were taken.

The chamber used to hold the solutions during irradiation was a cylindrical quartz vessel, 1.3 cm. high and 4 cm. outside diameter. The plane parallel ends were 2 mm. thick. The solutions were exposed from one of these ends. The liquid was poured in through a small quartz tube sealed to one side. The quartz chamber was immersed in a water bath to keep the solution temperature approximately constant. The water in the bath just reached the top face of the cell. A short rubber capsule was fitted tightly over the open end of the side tube to prevent evaporization and to keep the bath water from entering the chamber.

The solutions were irradiated with different parts of the spectrum of a mercury arc therapy lamp, which operated on 65 volts and 4 amperes d. c. Various glass filters were placed directly over the quartz chamber. The transmission of the filters: quartz, blue-purple Corex "A", clear Corex,* clear Corex "D" and window glass was measured by means of a quartz spectrograph and a recording

TABLE I.

% of Methylene Blue Faded by Rays from Different Parts of the Ultra-violet Spectrum.

.0058 mg. per cc. in water and 30% acetone.

Solution Temp.	Distance Inch.	Min. Exposure	Filters plus 2 mm. of quartz	10% or less Transmission	% Faded
			mm.	mμ	
26.6 C°	12	19	0	230	69.9
26.9	12	19	5 Blue-purple Corex “A”	258	28.2
27.2	12	21	6 Clear Corex	265	14.0
26.8	12	19	4 Clear ” “D”	290	1.2
27.0	12	19	2 window glass	321	0.0
			.0058 mg. per cc. in water		
27.5	12	165	0	230	51.4
27.8	12	165	5 Blue-purple Corex “A”	258	5.8
28.0	12	165	6 Clear Corex	265	0.0
29.2	12	165	4 Clear ” “D”	290	0.0
29.0	12	165	2 window glass	321	0.0
			.058 mg. per cc. in water and 30% acetone.		
28.5	12	160	0	230	92.9
29.0	12	387	5 Blue-purple Corex “A”	258	90.0
29.0	12	722	6 Clear Corex	265	93.6

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* This piece of clear Corex was obtained from the Department of Physiological Chemistry, University of Minnesota. Its exact classification is not known, but it is probably an old piece of clear Corex “A.”

micro-photometer. Their transmission thus obtained is in good agreement with the values commonly recited in the literature.

The results of a group of experiments are recorded in Table I. In column 5 are listed the maximum wavelengths of the ultra-violet spectrum which are transmitted by the filters by no more than 10%. For example, the combination, quartz and window glass, will transmit no more than 10% of the radiant energy of wavelengths shorter than 321 mμ. Two points of interest are demonstrated by the tabular values. First, the per cent of methylene blue in acetone changed,

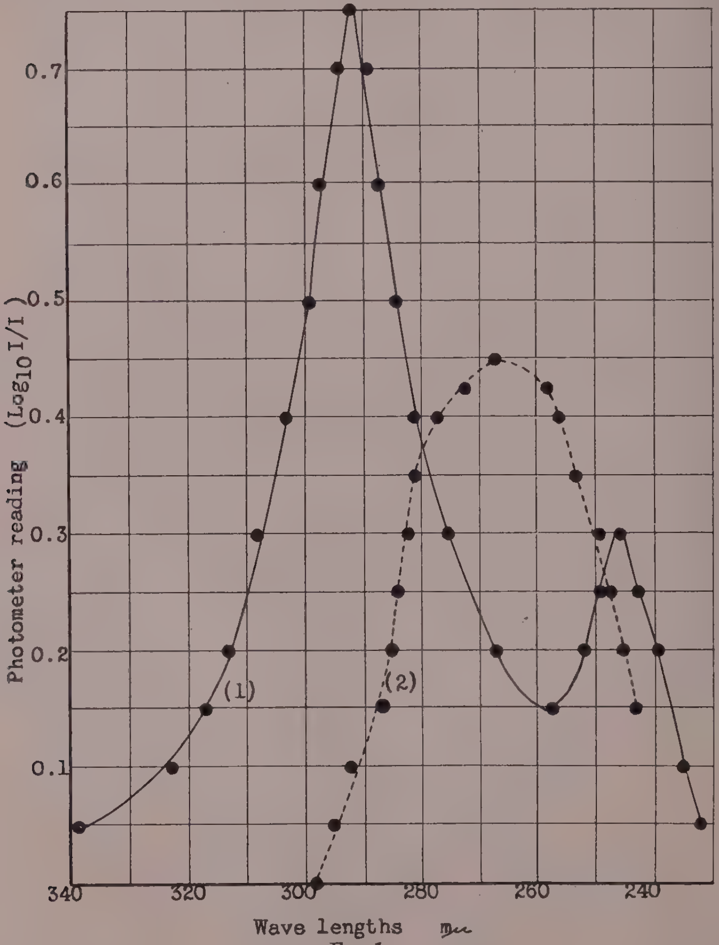

FIG. 1.

Absorption spectra of (1) methylene blue in water and (2) 0.2% acetone in water.

as determined by its absorption of visible light decreases rapidly when waves shorter than 290 mμ are absorbed by the filters. Second' the presence of acetone increases the effectiveness of wavelengths between 250 and 300 mμ to change the methylene blue. This apparent increase of sensitiveness of methylene blue to these rays when in the presence of acetone may be expected from an analysis of the absorption spectrum of the substances. Their absorption of ultra-violet light is shown in Fig. 1.† The pronounced absorption of acetone with a maximum at 267 mμ may result in the transfer of the absorbed energy from the acetone molecules to those of methylene blue, and in this manner increase the fading of the solution. The absorption of methylene blue around 290 mμ does not seem to contribute to the fading. It has been suggested by other investigators that acetone is decomposed into acetic and formic acids and that these acids produce the color change. This assumption, however, is not supported by the results of the following experiments: First acetic and formic acids were added to aqueous methylene blue and fading occurred promptly. Then acetone was irradiated under the conditions described previously and added to aqueous methylene blue. No more fading occurred than was expected by dilution alone. Therefore, it would seem that both acetone and methylene blue have to be present in the same solution at the time of irradiation in order to produce significant increases in the color change. It is of interest to note here that when a sample of .0058 mg. per cc. of methylene blue in 30% acetone was irradiated with X-rays practically no color change occurred. Irradiation of the same concentration in water without acetone under the same exposure conditions resulted in an almost colorless solution.

Ultra-violet rays longer than 290 mμ have an almost negligible effect as compared to rays shorter than 260 mμ. The "physiologically active" rays, usually designated as the region between 310 and 290 mμ, therefore, have an insignificant part in the fading of methylene blue by the light from a mercury arc lamp. Thus, a scale of skin erythemas in terms of units of color change of the solution would be subject to wide fluctuations. Since there is a skin erythemal effectiveness of wavelengths shorter than 270 mμ,[5, 6] any regular relationship between erythemal production and methylene blue fading is more likely to occur in the shorter wavelength region. It might be possible to establish some kind of scale between visible skin reactions and solution color changes by use of a filter which would absorb rays below 280 mμ.

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† The acetone curve agrees qualitatively with that of Victor Henri.

[5] Hausser, K. W., *Strahlentherapie*, 1928, **28**, 25.

[6] Luekiesh, M., Holladay, L. L., and Taylor, A. H., *J. Opt. Soc. Am.*, 1930, **20**, 423.

In the course of the work it was noted that solutions of methylene blue in acetone were not only faded by ultra-violet light but also were subjected to a color change from blue to green to a pale yellow. This series of colors became more apparent as the initial concentration of methylene blue was increased. The color change reversed when the solutions were removed from the ultra-violet light. If the green color predominated at the end of an exposure then the reverse change was rapid. Consequently, the solutions had to be transferred quickly from the irradiation chamber to the photometer cell and the match points read immediately. The yellow colored solutions, however, required several days to change from yellow to green to blue. The peculiar behavior of the color adds to the uncertainty of intensity determinations by this method. It is particularly true when fading is estimated in the simplest way; that is, when a comparison of the color intensity of the irradiated solution with a set of standard solutions of different concentrations is made without the help of any measuring instrument.

Summary. 1. The quantity of methylene blue in acetone solutions faded by ultra-violet from a mercury arc lamp decreases when the shorter wavelengths are absorbed by filters. 2. Addition of acetone to aqueous methylene blue solutions increases the effectiveness of the rays up to 300 mμ. 3. An aqueous solution of methylene blue and 50% acetone is not suitable for measurements of physiologically active ultra-violet radiation from an arc unless a suitable filter is used which reduces the intensity below 280 mμ. 4. Methylene blue acetone solutions undergo a color change from blue to green to yellow which reverses direction when the solutions are removed from the irradiation beam.

New York Section.

7444

Supplementary Note on Skin Sensitization with Simple Chemical Compounds.

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The following observations may be recorded as an addendum to the note[1] in a past issue of this journal. Further experiments were carried out with dinitrochlorbenzene[*] ($Cl:NO_2:NO_2 = 1:2:4$) which is known to be a frequent cause of trade eczema.[2] The reactions observed with this compound were rather stronger than those with nitroso-dimethylaniline. For instance, in one experiment lesions approximately 7 to 15 mm. in diameter were obtained after 18 intracutaneous injections on the back of 1/400 mg.[†] each, 2 a week for 9 weeks, when tested with the same concentration by intracutaneous injection on the side, one week after the last injection, and read on the following day.[‡] Reactions were noticed during the treatment, beginning with the third, fourth or later injections, and reaching a maximum after about 12 injections. Some sensitization appeared in practically all the guinea pigs injected, and the strongest reactions showed necrotic centers. In a second experiment the effects were weaker than in the above, although definite when tested one week after the fourteenth injection. Another batch receiving 10 daily injections and tested a week later exhibited strong reactions with livid centers in 3 animals and weaker reactions in the remaining 2. Two similar experiments yielded less prominent but positive effects.

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[1] Landsteiner, K., and Jacobs, J., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 790.

[2] Hamilton, A., Industrial Poisons in the United States, Macmillan, 1925, p. 500.

[*] Previously inaccurately designated as 1:2:4 dinitrochlorbenzene.

[†] Such small quantities could also be used for the treatment with nitroso-dimethylaniline.

[‡] Distinct reactions were also observed on guinea pigs applying 1 drop of a 1% solution of dinitrochlorbenzene in olive oil on the skin.

In cross tests with the 2 substances named a sharp specificity was evident since guinea pigs treated with dinitrochlorbenzene gave no distinct reaction to nitroso-dimethylaniline, and vice versa, as exemplified in Table I.

TABLE I.

Guinea pigs sensitized to dinitrochlorbenzene or p-nitroso-dimethylaniline, and tested with 1/400 mg. of the former and 1/50 mg. of the latter by intracutaneous injection of 0.1 cc. of solutions of the substances in 1% saline.§ The figures give the diameters in millimeters.

Treated with:		Tested with:	
		Dinitrochlorbenzene	Nitroso-dimethylaniline
Dinitrochlorbenzene	**1**	10.5, pale pink, elevated, livid center 3	almost negative
	2	11, pink, elevated, slightly livid center	3.5, pale pink, elevated
Nitroso-dimethylaniline	3	almost negative	8.5, pale pink, slightly elevated
	4	” ”	11, pink, elevated

The animals treated with p-nitroso-dimethylaniline did not give definite reactions with dimethylaniline, nitrosobenzene, or p-nitrosophenol, nor did the animals treated with dinitrochlorbenzene react to 2:4 dinitrophenol, o- or p-nitrochlorbenzene, m-dinitrobenzene, or picric acid.

Preliminary experiments with 2:4 dinitrophenol, which has been reported to produce hypersensitiveness in human beings,[3] gave suggestive but not quite conclusive results.

7445 P

Transplantation of Taste Organs in Adult Triturus Viridescens.

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From the Department of Anatomy, Yale University School of Medicine.

A study of the development of taste organs in the living condition in embryos and larvae of *Amblystoma punctatum*[1] has shown a considerable degree of independence of the organs with respect to their normal (gustatory) nerve fibers. In the present studies an attempt was made to examine the relationship between the taste organs and their gustatory fibers in the adult salamander. Because of the peculiar advantage of an aquatic host in which tongue grafts would be bathed constantly in a fluid environment, the common *Triturus viridescens* was used.

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§ An alcoholic solution of dinitrochlorbenzene was diluted with saline.

[3] Frumess, G. M., *J. Am. Med. Assn.*, 1934, **102**, 1219; Anderson, H. H., Reed, A. C., and Emerson, G. A., *J. Am. Med. Assn.*, 1933, **101**, 1053.

[1] Stone, L. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1256.

A small segment from the tip of the tongue was transplanted either to the side of the body or to the tail fin or into the orbit in place of the excised eye. One hundred and eight operations were made and with the exception of 2 cases all were autoplastic grafts. The operation was performed on chloretonized animals by means of fine iridectomy scissors and forceps manipulated under a compound dissecting microscope. Following the operation the animals remained quiet in moist chambers for 24 hours. They were then placed in water in individual glass aquaria and kept at room temperature.

The orbit with eyelids excised was found to be the most favorable site for the graft, for, in other places skin epithelium and body movements tended to interfere with early "takes". In 21 cases the graft was shed early or was later sloughed or completely resorbed. In the majority of transplants the circulation returned from 4 to 6 days after operation. This indicates how early the grafts are successfully healed in the new environment.

Edema of tissue underlying the epithelium of the graft mechanically distorted and flattened the epithelium and taste organs as early as the second day after operation. The edema then spread to the epithelium and some necrosis of cells took place. Leucocytes and macrophages made slight invasions as soon as circulation was reestablished. From the second to the twelfth day there was a progressive increase in mitotic activity in the epithelium. By the sixteenth and seventeenth days the taste organs had regained their normal appearance. In the few specimens killed from 18 to 28 days no sense organs could be identified. Whether these were exceptions or indications of a significant period in the change of organs following such an operation will not be clear until a larger number of animals are sacrificed during this time interval. Organ cells might have been present with no morphological characteristics to distinguish them from the surrounding epithelium. To us this seems more tenable than the possibility that all organ cells had completely degenerated, for grafts with similar histories in the living condition showed taste organs from 28 to 186 days after operation.

The results of these experiments show clearly that the taste organs can exist apart from the gustatory nerve fibers for at least as long as 6 months in the adult salamander. No special nerve stains have as yet been used. It is therefore not known whether or not other nerve fibers in the new environment have exerted any influence upon the taste organs.

No direct evidence was obtained of the formation of new organs by budding such as already found in grafted tongues of larvae.[1] The tongue epithelium of the adult is too thick and the taste organs are too deeply imbedded to allow a satisfactory study of the living graft under the high powers of the microscope. However, during and after the proliferative stage of the epithelium mitotic figures were found in some organs. The size of the graft did not increase with the advance of age. As a matter of fact there was some decrease. Nevertheless, the older grafts showed many more organs than those at the time of operation or shortly after. This may indicate that taste organs increase in numbers in adult tongues by a budding process such as is found in larvae.

7446 P

Grafted Eyes of Adult Triturus Viridescens with Special Reference to Repeated Return of Vision.

L. S. STONE, I. S. ZAUR, AND T. E. FARTHING.

From the Department of Anatomy, Yale University School of Medicine.

The present experiments are part of a series of investigations which have already been reported[1, 2, 3, 4] on grafted larval and adult amphibian eyes. Over 600 grafts of functional eyes have been studied in various types of operations. All eyes while living have been observed daily and the findings have been correlated with closely staged histological preparations.

This report is based on the observations of 140 reimplanted and transplanted eyes in the adult *Triturus viridescens.* In all cases the eye has been placed in the orbit, *i. e.,* in its normal environment. In some cases the eyes have been regrafted as many as 2 and 3 times involving reimplantation and transplantation in different orders of sequence.

Following operation some reduction in the size of the eye takes place as in the case of larval eyes. The latter recover their original size after the return of circulation and soon become equal to that of the normal control. In adult eyes the reduction in size is maximally attained at a time (20-25 days) when the degeneration of the retina has reached its height. In most cases there is no complete recovery in the size of the eye although in other respects it may appear normal except for slight histological changes.

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[1] Stone, L. S., and Ussher, N.T., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 213.

[2] Beers, D. N., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 477.

[3] Stone, L. S., *J. Exp. Zool.*, 1930, **55**, 193.

[4] Stone, L. S., and Cole, C. H., PROC. SOC. EXP. BIOL. AND MED., 1931, **29** 176.

Observed return of circulation in the superficial vessels of the iris usually occurred within the first week after operation, the earliest being on the fifth day. However, temporary opacities of the cornea in some cases prevents the observation of the earliest movement of blood cells within the vessels.

Slight or even considerable opacity of the cornea took place in about half of the cases. Except in a few sloughed eyes this condition was transient and usually disappeared in a few days. Histological examination showed edema and thickening of the cornea. The changes were largely in the *substantia propria* which by hypertrophy brought about a permanently thicker cornea.

The lens undergoes degeneration very rapidly but before this is completed a new lens regenerates from the pupillary margin of the iris. This is as early as the nineteenth day after operation. The new lens appears structurally normal in less than 4 weeks after operation but for at least 4 or 5 months the lens never attains the size of that of the normal.

There is no measurable pupillary response to light and darkness in the adult eyes of this species such as there is in Amblystoma. However, following operation there is an early rapid reduction in the size of the pupil in all cases. The maximum reduction is attained during the period of degeneration seen in the retina. During the regeneration stages, beginning about the third to fourth week, the pupil begins to widen but never in 4 or 5 months attains the diameter equal to the original. The pupil may even in some cases fluxuate in size before it reaches a point of stability.

A marked degeneration of the retina takes place by the second day after operation and progresses until, by about the end of the third week, it is destroyed with the exception of the ciliary margin. From this margin regeneration extends as a single layer of cells, and, by rapid proliferation various layers of a new retina begin to appear as early as the thirty-fifth day after operation.

The degeneration of the optic nerve follows about as rapidly as the retina. Its complete regeneration coincides with the differentiation of the new retina. A complete connection with the brain is made before the end of the second month and by the third month return of vision is clearly demonstrated.

A selection of 35 eyes was made for the tests of visual function and in every case proof of return of vision was obtained between the second and third month after operation. The technique has been reported in the literature cited. One case will give an idea of the results after grafting the eye 3 times (reimplant, reimplant, transplant, respectively). The first return of vision was on the eighty-seventh day; the second return of vision was on the seventy-ninth day; the third return of vision was on the sixty-first day. How many times this perfect eye could have been grafted so successfully is problematical.

Repeated grafts showed that the eye went through the same changes each time as in the first operation. There was only one striking change and that was a gradual permanent reduction of the ganglion cell layer, about 40% reduction. We hope to use this method in testing how far the reduction of ganglion cells can be obtained and still prove the presence of visual function.

7447 P

Production and Metamorphosis of Chimeras in Anurans and Urodeles.

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The perfect union of the anterior and posterior halves of the embryos of 2 species of anurans[1] and the metamorphosis[2] of such a combination into a perfect adult was shown to be possible by Harrison 30 years ago. Up to the present day the opportunities which this operation makes possible have never been realized. The intention of the present report is to demonstrate that such combinations can be produced in large enough numbers to enable the experimental embryologist and geneticist to carry out a variety of new studies.

The frog embryos employed were *Rana palustris* and *Rana sylvatica*. Those of the salamander used were *Amblystoma tigrinum* and *Amblystoma punctatum*. The stages at which the operation was done were in the early tail-bud period of development.

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[1] Harrison, R. G., *Arch. f. mikr. Anat. u. Ent.*, 1904, **63**, 35.

[2] Harrison, R. G., *Anat. Rec.*, 1908, **2**, 385.

The species conbinations in their respective classes are, at the tail-bud stages, about the same in size. Similar stages in development were selected and placed in slightly diluted Ringer's solution in an operating dish, the floor of which was covered by a thick film of white beeswax. The embryo was oriented under the microscope, dorsal side up, between fine forceps so that iridectomy scissors could, with one stroke, cut the embryo transversely at the level of the seventh to eighth somites. It is quite possible to cut at other levels as well in making successful combinations.

The anterior half of one species and the posterior half of the other were then placed in a depression in the wax which was about the size of an intact embryo. The cut surfaces of the reciprocal halves were brought together in the concavity of the wax as soon after the operation as possible.

When the incision is made with one stroke the lateral ectodermal surfaces come together and temporarily seal in the mid line. This prevents the immediate escape of the heavily laden yolk cells. The ectodermal edges then slowly separate and by the time the reciprocal halves are properly oriented the cut surfaces are closely opposing one another.

By means of the flat broken end of a fine glass rod finger-like spurs of wax can be produced to form a tight cage over the 2 halves of the embryos in the depression. This is done by pushing the end of the glass rod in the surface of the soft wax from the periphery of the depression toward the position occupied by the embryonic halves. Each finger-like spur of wax increases to the desired length as it is shoved nearer the desired spot. Furthermore the projections of wax curl in such a manner that they form flat bands which incase the 2 embryonic halves. The spurs of wax are flexible enough so that each can be adjusted to the proper position as desired.

With but little experience the operator can orient 6 wax bands, 2 on either side and 1 at the anterior and posterior ends, respectively. These are sufficient to unite the embryonic halves, permitting healing within 2 hours.

The halves of the embryos were oriented with the dorsal surfaces uppermost so that one could be certain that the cut ends of the neural canals were perfectly apposed for proper healing. Some yolk laden cells ooze from the cut surfaces. This is more marked in the urodeles than in the anurans. Very often the ventral surfaces are not entirely healed when the chimera is lifted from the wax depression. Therefore, great care must be exercised at this point, for a large ventral wound may cause the early death of the embryo or later on lead to the formation of a permanent fistula.

In one series of 16 anuran combinations, 12 reached late larval life and of these 8 metamorphosed into frogs. Each showed by the skin coloration the regions of the 2 species in combination. In another series of 15 combinations 7 are now in late larval life and one has metamorphosed. Four of these consist of 2 perfect reciprocal pairs.

In one series of 9 pairs of urodele combinations 3 have metamorphosed and one is approaching metamorphosis. In another series of 9 pairs, 3 are in mid-larval life. All of these are perfect specimens. Several were imperfectly united and lived to mid and late larval life when they were discarded.

The head, thorax and forelimbs of the anterior half of the combination attained the normal size and appearance of the species it represented. It, however, exerted a marked influence over the posterior half of the combination. The normally smaller anterior half inhibited the growth of the normally larger posterior half. The normally larger anterior half reversed the conditions by increasing the size of the normally smaller posterior half. In other words, the anterior half was always species dominant in its effect. This was very striking in the late larval and early adult life.

7448 P

Glucose Metabolism of the Trypanosoma Equiperdum in Vitro.

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It has previously been found that trypanosomes can live either aerobically or anaerobically. The oxygen consumption has been measured and in some instances also compared with the glucose utilization.[1, 2, 3, 4] In the present paper a report is given of measurements of the glucose and oxygen consumed and the products formed aerobically and anaerobically.

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[1] Yorke, W., and Nauss, R. W., *Ann. Trop. Med.*, 1912, **5**, 199.

[2] Fenyvessy, B. v., and Reiner, L., *Z. Hyg.*, 1924, **102**, 109; *Biochem. Z.*, 1928, **202**, 75.

[3] Issekutz, B. v., *Arch. Path. u. Pharmakol.*, 1933, **173**, 479.

[4] Brand, T. H. v., *Z. vergleich. Physiol.*, 1933, **19**, 587.

The first set of experiments in which the oxygen consumed was compared with the total acid produced was carried out using the Barcroft-Warburg apparatus. The medium consisted as a rule of glucose dissolved either in Ringer solutions equilibrated with CO_2 or in isotonic phosphate buffer solutions, pH 7.4. It was found in experiments with phosphate buffer that the CO_2 produced is comparatively small. Hence, the CO_2 found liberated from bicarbonate solutions was chiefly due to the production of a non-volatile acid. The ratio of the mols acid produced to the oxygen consumed was fairly constant in all experiments and averaged 1.74 ± 0.07.

In another set of experiments the fixed acid produced was determined by titration with NaOH and compared with the glucose utilized, as determined by the Hagedorn-Jensen method.[5] The ratio of the mols of acid produced to the mols of glucose consumed was again near to 2, *viz.,* 1.80 ± 0.22 in aerobic experiments and near to 1, *viz.,* 0.87 ± 0.11, in the anaerobic experiments.

Lactic acid determinations by the Friedemann, Cotonio and Shaffer method[6] showed that only a negligible fraction of the non-volatile acid is lactic acid.* The fact that in aerobic experiments the ratio of mols acid produced to mols glucose consumed was near to 2 and that nearly 2 equivalents of acid were produced per mol oxygen consumed, suggested that 2 molecules of pyruvic acid or an isomer thereof, were produced by oxidation of one molecule of glucose. Indeed, the presence of pyruvic acid in the solutions was shown by the isolation of its phenylhydrazone. In more quantitative experiments 53-75% of the titratable acid was isolated and identified by mixed melting point as the phenylhydrazone of pyruvic acid. The acid produced under anaerobic conditions was again chiefly pyruvic acid. Fifty-two to 72% of the titratable acid was again recovered and identified as the phenylhydrazone of pyruvic acid. If one molecule of glucose is split to form one molecule of pyruvic acid, the empirical formula of the substance which remains is that of glycerol. This same empirical formula requires one molecule of oxygen for conversion to pyruvic acid.

It has been reported that glycerol is able to "revive" trypanosomes which have lost their motility because of lack of food.[7] We found that this "revival" takes place only under aerobic conditions. Trypanosomes suspended in glycerol containing buffered salt solution lose their motility in a very short time if kept anaerobically. Studies of the glycerol metabolism under aerobic conditions were made by the same methods as described above for glucose. The amount of total acid produced was, here, 1 mol per 1 mol of oxygen. No appreciable amounts of CO_2 were found. The presence of pyruvic acid was demonstrated by the action of yeast carboxylase and most of the titratable acid was recovered as the phenylhydrazone of pyruvic acid.

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[5] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

[6] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

* Traces of lactic acid may be produced by glycolysis by red cells. The trypanosomes were obtained by fractional centrifugation from an infected rat's blood, and as a rule a small number of red cells were present.

It remained to be seen whether or not the non-acid substance produced by trypanosomes anaerobically from glucose actually is glycerol. Determinations of glycerol according to the method of Zeisel and Fanto[8] showed that, in emulsions of trypanosomes containing glucose and kept anaerobically, this substance was present in amounts approximately equal to the amount of acid found. The average result of 3 experiments was 0.87 ± 0.11 mols acid and 0.97 ± 0.24 mols of glycerol per mol of glucose.

Preliminary experiments indicate that the presence of serum does not alter the described course of glucose metabolism. The evidence presented here for the production of large amounts of non-volatile acid agree with the fact that a decrease of the alkaline reserve is one of the symptoms of the experimental *Trypanosoma equiperdum* infection of rats.[9]

Summarizing, the following scheme is suggested for the main path of glucose utilization by trypanosomes *in vitro:*

$$\text{Glucose} \longrightarrow \text{Glycerol} + \text{Pyruvic Acid}$$

$$\text{Glycerol} + O_2 \longrightarrow \text{Pyruvic Acid} + 2\ H_2O$$

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[7] Kudicke, R., and Evers, R., *Z. Hyg.*, 1924, **101**, 317.

[8] Described in Allen's Commercial Organic Analysis, 5th ed., Vol. II, 666.

[9] Kligler, I. J., and Geiger, A., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 229; Kligler, I. J., Geiger, A., and Comaroff, R., *Ann. Trop. Med.*, 1929, **23**, 325; Scheff, G., *Biochem. Z.*, 1928, **200**, 307; Linton, R. W., *J. Exp. Med.*, 1930, **52**, 103, 695.

7449 P

Costal and Abdominal Respiratory Movements in Relation to Nervous Control of Breathing.

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Changes in body circumference associated with respiratory movements were simultaneously recorded in the dog at 6 different levels—3 costal and 3 abdominal. (Fig. 1.) Continuous registration of respiratory movements with this method showed variations in type from time to time in the same individual. Some of these changes were spontaneous; others were elicited by deliberate introduction of new variables.

Analysis of measurements on 31 female and 73 male dogs failed to show any relation between sex and mode of breathing. Comparing the magnitude of increased costal circumference with the magnitude of increased abdominal circumference 34.3% of 106 dogs were found to breathe more with the chest than with the abdomen. Using our new method of comparing costal and abdominal accommodation of air by grossly correcting for the greater costal circumference and greater length of chest 79% were found to be costal breathers. The mean ratio of costal to abdominal accommodation for all of the dogs studied was 2.15 indicating a decided predominance of costal breathing in the dog.

A shifting from a more costal or more abdominal type towards the other respective type was frequently preceded by a momentary and sporadic stretching of the extremities. At times, periodic fluctuations in respiratory movements occurred, limited to either costal or abdominal levels. These findings suggested that periodicity may be a localized phenomenon dependent on chemical sensitivity in the cord.

Great irregularities in the magnitude of respiratory excursions occurred mostly from changing inspiratory circumference. Under these conditions the expiratory circumference may remain surprisingly uniform. In some experiments the expiratory circumferences changed as well. At times the expiratory circumference of the chest showed gross fluctuations while those of the abdomen remained perfectly constant. The reverse also occurred.

The magnitude of costal and abdominal expansion in sporadic deep breaths varied out of proportion to the prevailing respiratory movements. The deep breaths were, therefore, frequently of opposite type to the prevailing type.

Backward injection of sodium citrate into the femoral artery produced differential effects in costal and abdominal breathing. Intravenous injection of urethane inhibited costal breathing more than abdominal.

Lowered alveolar oxygen most commonly increased the circumference of the chest and abdomen at the end of expiration and decreased the intrathoracic pressure. During recovery, costal respiratory movements were inhibited more than abdominal respiratory movements. The upper costal segments were inhibited more than the lower costal segments. Upper costal apnea and abdominal hyperpnea were not uncommon during recovery. In one experiment upper costal apnea was accompanied by abdominal hyperpnea greater than the hyperpnea of the immediately preceding anoxemia. As abdominal respiratory movements diminished during recovery the costal movements increased. This unusual coordination(?) of costal and abdominal breathing led to a smooth return to normal ventilation. (Fig. 1.)

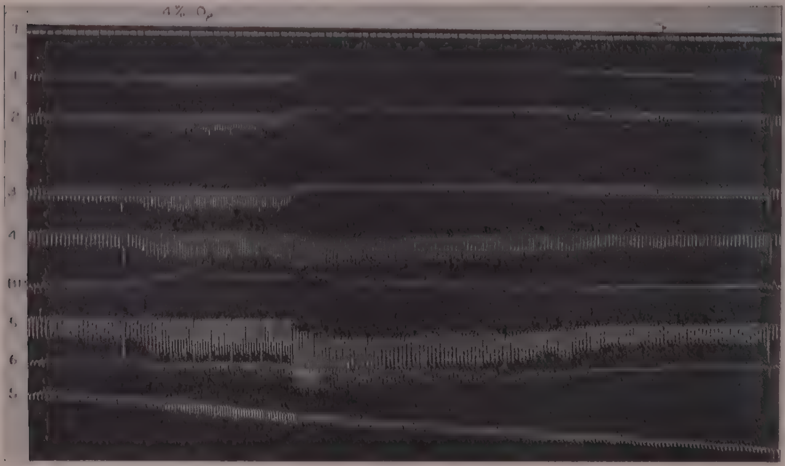

FIG. 1.

The effects of carbon dioxide differed importantly from those of anoxemia. In most experiments there was a decrease in circumference of the chest and abdomen at the end of expiration and a corresponding rise in the intrathoracic pressures.

Inasmuch as costal or abdominal breathing may shift from one to the other it is concluded that all dogs are provided with mechanisms permitting either type or any combination of types of breathing.

To determine whether the level of entrance in the central grey axis stem of sensory nerve impulses exerts a localized effect on breathing, such nerves as the saphenous, radio cutaneous, infra orbital and phrenic were stimulated faradically. A comparable response of accelerated breathing, decreased costal expiratory circumference and a tendency towards increased abdominal circumference was obtained in each one.

The effects of graded sensory stimulation of the mixed cervical vagus and of the pulmonary branches of the same differed considerably. These differences were attributed to the abundance of extra pulmonary nerve fibers in the cervical vagus nerve.

A strength of stimulation which will produce pure inhibition when applied during expiration may increase the depth of one introductory inspiration if applied during the phase of inspiration. The accessibility of the center to excitatory and inhibitory influences therefore varies with the phase of the respiratory cycle.

Rhythmic breathing produced by rhythmic stimulation of the cervical vagus, pulmonary branches of the vagus, and the saphenous nerve indicate that the respiratory center may become accessible to rhythmic impulses from many sources agreeing with the synchronization of breathing and rhythmic muscular movements.

Rhythmic stimulation of the vagus nerve may also produce complete respiratory inhibition. Faradic stimulation of the saphenous nerve, or chemical excitation of the carotid gland with sodium sulphide or cyanide removes the inhibition and allows the animal to breathe with each vagal stimulation. When rhythmic stimulation of the vagus nerve produces rhythmic breathing the action is, most frequently, locally selective. Costal breathing is partly or completely inhibited and abdominal breathing is usually augmented. Under these conditions cyanide or sulphide lead to powerfully augmented costal respirations and less markedly augmented addominal respirations.

A higher pulmonary ventilation is attained by cyanide during double vagal block when the vagus nerves are rhythmically stimulated, which suggests the importance of normal vagal excitation produced by inflation and deflation of the lungs.

Faradic stimulation of the saphenous nerve, and intravenous injection of sulphide or cyanide produce much greater and more rapid ventilation with the vagus nerves intact than during vagal block, clearly indicating the great importance of the vagus nerves in the control of breathing.

These findings show the dependence of vagal function on inflowing afferent nerve impulses and further strengthen the general principle that breathing may be largely a resultant of numerous and various afferent nerve impulses.

7450 C

Reduction of Selenites and Tellurites by the Sulphydryl Group.

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The reducing effect of compounds containing the sulphydryl group has been tested with sodium selenite and with sodium tellurite as indicators. The following test solutions were prepared: Reagent (1), an acid reagent made up of 100 cc. of 2% sodium selenite or sodium tellurite plus 20 cc. concentrated hydrochloric acid; reagent (2), 2% sodium selenite or sodium tellurite solution, which is slightly alkaline; reagent (3), a more alkaline reagent containing 2% sodium selenite or sodium tellurite in 10% sodium carbonate. The compounds studied were the thio compounds, ethyl mercaptan, thioacetic acid, thioglycollic acid, thiocresol, thiourea, glutathione, cysteine hydrochloride, and the sulphur containing compounds not possessing the SH group—cystine, and thiophene.

On reduction sodium selenite yields free selenium in the form of a brick red precipitate or a red to brick red colloidal solution depending upon the intensity of the reaction and the quantity of the free element liberated. Sodium tellurite under similar conditions yields a brownish black precipitate of free tellurium or a black brown colloidal solution of this element. Compounds that reduce in the cold give more profuse reduction under the application of heat. The test is carried out by allowing 3 cc. of the compound in aqueous solution to interact with 3 cc. of the selenite or tellurite reagent. The reduction of sodium selenite or tellurite by the compounds is of special value, since the reaction is irreversible and the reduced selenium or tellurium does not display a tendency to undergo re-oxidation.

All the thio compounds examined with the exception of thiourea have very marked reducing capacity. The limits of sensitivity are given in Table I.

TABLE I.

Compound	Acid Reagent (1)		Alkaline Reagent (2)		Alkaline Reagent (3)	
	Cold	Heat	Cold	Heat	Cold	Heat
			Selenite Reagents.			
Ethyl mercaptan	negative	0.010%	0.30%	0.050%	0.30%	0.050%
Thioacetic acid	,,	0.005	0.08	0.070	0.08	0.800
Thioglycollic acid	,,	0.001	0.10	0.006	0.04	0.003
Thiocresol	,,	0.003	0.03	0.005	0.30	0.010
Glutathione	,,	0.008	0.10	0.020	0.07	0.010
Cysteine HCl	,,	0.020	0.03	0.030	0.05	0.050
			Tellurite Reagents.			
Ethyl mercaptan	0.09%	0.030%	0.30%	0.070%	0.30%	0.070%
Thioacetic acid	0.08	0.009	0.10	0.007	0.10	0.008
Thioglycollic acid	negative	0.001	0.07	0.003	0.09	0.003
Thiocresol	0.04	0.004	0.04	0.005	0.03	0.010
Glutathione	negative	0.009	0.25	0.080	0.04	0.040
Cysteine HCl	,,	0.090	0.03	0.030	0.04	0.040

The acid selenite reagent is not reduced in the cold by any of the thio compounds. The acid tellurite reagent is reduced in the cold by ethyl mercaptan thioacetic acid and thiocresol, but not by thioglycollic acid, glutathione, and cysteine. On heating, however, all the thio compounds reduce the acid selenite and the acid tellurite reagents. All the thio compounds reduce at room temperature and at the temperature of the water-bath the slightly more alkaline selenite and tellurite reagents.

Reduction in the cold takes place after a slight lapse of time. Two minutes we found the maximum time necessary for a precipitate to form. As the concentration of the thio compound is lowered a reddish colloidal solution forms but the time of its appearance is prolonged. A series of reaction mixtures may be made with different concentrations of thio compound as a classroom demonstration of reaction time.

The thio compounds are the only organic compounds that we have thus far studied with the exception of pyruvic acid, glucoreductone, and ascorbic acid that have such marked reducing properties that they reduce even at room temperature. Pyruvic acid reduces in the cold the 2% sodium selenite or sodium tellurite reagent containing 10% sodium carbonate. On the application of heat pyruvic acid reduces all the other selenite reagents and the acidified tellurite reagent. Pyruvic acid may be differentiated from the thio compounds by failure to reduce in the cold the slightly alkaline or the acidified selenite solution.

Glucoreductone reduces the highly alkaline selenite and tellurite reagents in the cold, but the slightly alkaline and the acidified selenite and tellurite reagents only on heating. Glucoreductone was prepared by heating a 5% solution of glucose in 2% sodium hydroxide and neutralizing the mixture with hydrochloric acid. The term reductones has been given by von Euler and Klussmann[1] to the highly reducing materials obtained when alkaline sugar solutions are heated. Kertesz[2] has recently advised the use of glucoreductone for the standardization of 2,6-dichlorophenol-indophenol solutions used for the estimation of ascorbic acid.

Ascorbic acid* reduces in the cold only the acidified selenite and tellurite reagents, but the application of heat brings about reduction in the slightly alkaline and in the more highly alkaline selenite and tellurite reagents. Ascorbic acid may be readily differentiated from glucoreductone and from the thio compounds. Ascorbic acid reduces in the cold the acidified selenite reagent, while glucoreductone and the thio compounds do not.

The sulphur-containing compounds—cystine, thiophene, and thiourea do not reduce. Thiourea possesses the sulphydryl group. Old and slightly discolored samples of thiourea possess some reducing power, but not the fresh compound. Cystine can be differentiated from cysteine, since the latter has marked reducing properties while the former has none.

Sodium selenite and sodium tellurite are very sensitive reagents for the detection of thio compounds including cysteine and glutathione. Thio compounds with the exception of thiourea can be differentiated from all other reducing compounds we have thus far studied[3] on the basis of the fact that the former react in the cold, that is at room temperature, with all the 3 selenite reagents. On the other hand, the thio compounds may be utilized in the detection of selenium in the form of selenite and of tellurium in the form of tellurite. They may also be employed in quantitative procedures where selenium or tellurium is estimated by weight in the element obtained by precipitation with a reducing agent. Cysteine HCl is especially adapted for this purpose, since it tends to give precipitates rather than colloidal solutions of selenium or tellurium.

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[1] von Euler, H., and Klussmann, E., *Ark. Kemi, Mineral. o. Geol.*, 1933, **11B**, Nos. 8, 11, 12.

[2] Kertesz, Z. I., *J. Biol. Chem.*, 1934, **104**, 483.

* We are indebted to Dr. C. G. King, of the University of Pittsburgh, for the sample of pure ascorbic acid.

[3] Levine, V. E., *Biochem. Bull.*, 1915, **4**, 217; *Science*, 1920, **52**, 391.

7451 P

Acceleration of Blood Coagulation by Breathing Oxygen.

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While conducting experiments to determine the rate of elimination of nitrogen from the body, in which the subject, either dog or human, breathed oxygen (99.5%) at atmospheric pressure; it was noted that the velocity of coagulation of the blood was greatly accelerated. During these experiments we have found it almost impossible to draw blood samples with a syringe without the use of an anticoagulant when the subject has been breathing oxygen.

In experiments where the femoral artery and vein of anesthetized dogs were exposed so that blood samples could be drawn at definite intervals, severe hemorrhage from injury to the artery occasionally occurred. When the animal was breathing room air it was necessary to clamp off the artery to stop bleeding. However, if the animal had been breathing oxygen for 15 minutes or longer the vessel repaired itself by external clotting and no clamping was necessary.

Further studies on the control of hemorrhage by breathing oxygen are in progress.

7452 P

Unidentified Gram Positive Bacillus Associated with Meningo-Encephalitis.

CASPAR G. BURN. (Introduced by Raymond Hussey.)

From the Department of Pathology, Yale University School of Medicine.

An unidentified gram positive bacillus has been procured from the blood stream of 3 newborn infants that died, having lesions determined at necropsy to be meningo-encephalitis during the past year and a half at the New Haven Hospital. The organisms were obtained during the clinical course of the disease of 2 of the infants and in all 3 instances they were isolated from the heart blood at postmortem. In 2 of the infants a routine bacteriological study of the organs at necropsy showed them to be diffusely distributed throughout the body. The organisms are pathogenic for rabbits, guinea pigs, mice and to a lesser degree young monkeys. They have an especial affinity for the tissues of the central nervous system.

The organisms are morphologically a small non-spore forming gram positive bacillus measuring 1 to 4 micron in length and 0.5 micron in breadth with an occasional tendency to form longer rods or filaments. They are usually arranged singly or in small clumps with some tendency to palisade formation. All strains are non-acid fast. The colony on a blood agar plate is round, moist, convex and semi translucent. It is not quite as opaque or dense as colonies of *Streptococcus hemolyticus*, although it is necessary to make a very careful morphological study of the organism for they may very easily be mistaken for strains of hemolytic streptococci because of the colony appearance and their marked hemolytic properties on blood agar plates. Hemolysis occurs both on surface and deep pour blood agar plates, as well as a rapid hemolysis takes place in broth of both rabbit and human red blood corpuscles. The strains grow readily and rapidly in meat infusion broth without aid of further nutritive material. They ferment dextrose, maltose, salicin, dextrin, galactose, starch and rhamnose in 24 hours, while lactose and glycerin fermentation is delayed usually for 48 to 72 hours. Litmus milk is slowly acidified and decolorized, but is not coagulated. Gelatin is not liquefied. They are very sluggishly motile, but it is extremely difficult to differentiate true motility in all the cultures studied. It fails to form indol or reduce nitrates. All 3 strains are very hardy and survive in ordinary media for long periods of time without transfer.

Serological studies of the 3 organisms demonstrate that they are homogeneous strains. Because of their resemblance in morphological and cultural properties to *Bacterium mononcytogenes*[1] further work is now in progress for studying this relationship.

When young rabbits were inoculated intravenously with known quantities of this organism (1 cc. volume) the clinical course of the disease and the pathology varied with the number of bacilli inoculated. Six animals receiving 500 million to 1 billion bacilli per cc. died within 36 to 48 hours from an overwhelming infection. In 27 rabbits receiving 6000 to 30 million bacilli per cc. there were 17 that developed signs of central nervous system involvement and at postmortem showed an extensive meningo-encephalitis. The liver and spleen contained small foci of necrosis. Three of the remaining

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[1] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.*, 1926, **29**, 407.

10 animals that survived showed evidence of meningitis when they were sacrificed.

The site of the inoculation of the organism into the rabbits determines the course of the disease process. If small quantities of organisms are inoculated into the cisterna, the rabbits died within 24 to 48 hours with an extensive meningo-encephalitis. On the other hand, intramuscular or intraperitoneal injections did not produce any apparent change, either clinically or anatomically.

Mice, guinea pigs and young monkeys were found to be susceptible to intravenous inoculation of the organisms.

In summary, there has been isolated from the blood stream of 3 newborn infants an unidentified gram positive bacillus that is found to be pathogenic for the usual laboratory animals and apparently has a predilection to localize in the central nervous system when given intravenously in small quantities.

7453 C

Validity of Iodine and Copper Reduction Methods for Amylase.

LEON C. CHESLEY. (Introduced by P. S. Henshaw).

From the Department of Zoology, Duke University, Durham, N. C.

If a quantitative method for the determination of amylase be valid and practical, different samples of the substrate—soluble starch—must give similar and reproducible results.

In a previous paper[1] the Wohlgemuth and viscometric methods were investigated by comparing the amounts of enzyme required to produce a given result in different samples of soluble starch. Both of these methods measures the dextrinogenic constituent of amylase; the Wohlgemuth was found to be non-valid as a quantitative procedure, while the viscometric method seems to be valid if properly controlled. Thompson, McGarvey and Wies[2] have obtained good results with the viscometric method by mixing together different samples of soluble starch.

The purpose of the present paper is to investigate the iodine and copper reduction methods as indicated above; the latter method is for the measurement of the saccharogenic agent of the amylase complex.

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[1] Chesley, L. C., *J. Biol. Chem.*, 1931, **92**, 171.

[2] Thompson, W. A., McGarvey, S. M., and Wies, C. H., *J. Gen. Physiol.*, 1932, **16**, 229.

A. The achromic iodine method and variations.

The first procedure used was that devised by Johnson.[3] The determinations were conducted exactly as specified in his paper; no sodium chloride or phosphate buffer were used. The substrate was a 2% soluble starch preparation; correction was made for the moisture content.

Into each of a series of flasks in a water bath at 40°C. were placed 50 ml. portions of the soluble starch, which was allowed to come to the temperature of the bath. At 10 second intervals, successively increasing amounts of dilute saliva were added to the flasks. (Different specimens of human saliva diluted with different volumes of distilled water were used.) At the end of 10 minutes, 5 drops were removed from the digest and added to 5 ml. of N/8000 iodine solution. The first tube in which no color was developed was taken as the end-point. The quantity of enzyme solution which would just digest the starch to the end-point was determined as accurately as was practicable. The figures given in Table I under "ml. enzyme solution" represent the quantity of dilute saliva required to hydrolyze the soluble starch to the end-point in 10 minutes.

Two soluble starch samples were used simultaneously and under identical conditions. Sample 1 was Baker's analyzed C. P. soluble starch; Sample 2 was Lintner's soluble starch.

As shown in Table I, Series 1, Sample 1 required about 25% more enzyme than did Sample 2 to be digested to the same end-point in the same time. In Series 2, 3, and 4 (Table I) the effects of added chloride and phosphate buffer, both separately and together, are shown. The added sodium chloride was 0.05 M in final concentration, as was the phosphate buffer mixture, the pH, in the buffered series, was 6.9.

In all 3 cases, Sample 2 required about 45% more enzyme to be digested to the same end-point as Sample 1. It is seen that the relative digestibility is the reverse of that in the first series in which no electrolyte was added. The effect is not caused by a pH change, since Series 3 shows the same reversal as do Series 2 and 4. It may be caused by the presence of a greater quantity of activating electrolyte impurity in Sample 2.

Phosphates or chlorides, present as impurities in different amounts in the samples, are not responsible for the discrepancy in "digestibility" which exists between the 2 samples, since in Series 4 enough

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[3] Johnson, W. A., *J. Am. Chem. Soc.*, 1908, **31**, 798.

TABLE I.

Digestion of soluble starch Samples 1 and 2, as influenced by added electrolytes. Six experiments with different specimens of saliva.

Disparity in "digestibility" of samples indicated by quantities of enzyme solution required for hydrolysis to end-point.

Series 1
Unbuffered
(1), pH 6.5; (2), pH 6.0
Added phosphate, none
Added NaCl, none
				Series 2
				Buffered
				pH 6.9
				Added phosphate, .05 M
				Added NaCl, none			

Exp.	Ml. enzyme solution Sample 1	Ml. enzyme solution Sample 2	Ratio	Exp.	Ml. enzyme solution Sample 1	Ml. enzyme solution Sample 2	Ratio
1	1.20	1.00	1.20:1	1	1.20	1.60	.75:1
2	1.20	1.00	1.20:1	2	1.00	1.60	.62:1
3	0.80	0.60	1.33:1	3	1.10	1.50	.73:1
4	0.80	0.60	1.33:1	4	0.80	1.20	.67:1
5	0.80	0.60	1.33:1	5	0.80	1.20	.67:1
6	0.80	0.70	1.14:1	6	0.70	1.00	.70:1
	Mean		1.25:1		Mean		.69:1

Series 3 Unbuffered (1), pH 6.5; (2), pH 6.0 Added phosphate, none Added NaCl, .05 M				Series 4 Buffered pH 6.9 Added phosphate, .05 M Added NaCl, .05 M			
Exp.	Ml. enzyme solution Sample 1	Ml. enzyme solution Sample 2	Ratio	Exp.	Ml. enzyme solution Sample 1	Ml. enzyme solution Sample 2	Ratio
1	.40	.65	.61:1	1	.15	.225	.67:1
2	.40	.60	.67:1	2	.20	.275	.72:1
3	.40	.60	.67:1	3	.15	.250	.60:1
4	.40	.60	.67:1	4	.20	.250	.80:1
5	.40	.60	.67:1	5	.20	.300	.67:1
6	—	—	—	6	.30	.400	.75:1
	Mean		.65:1		Mean		.70:1

of both chloride and phosphate was added to render negligible the differences in concentrations of these contaminations.

B. The copper reduction method.

In the present investigation, the Walker-Munsen and Bertrand methods were combined for the estimation of maltose (Mathews[4]).

Two percent soluble starch was prepared, sodium chloride and phosphate buffer was added to 0.05 M each, and the pH was maintained at 6.9; the temperature was held at 35°C. To 25 ml. portions of the soluble starch was added one ml. of diluted saliva. At the end of 30 minutes, 5 ml. specimens were removed from the digest and run into an excess of Fehling's solution (25 ml. diluted to 95 ml.). The solution was kept in a bath at 112°C. for exactly 6 minutes, then removed and filtered through a Gooch crucible using suction. The precipitate was washed several times with distilled water at 60°C., then dissolved in M/2 ferric ammonium sulphate-sulphuric acid mixture and titrated at once with N/20 potassium permanganate. Two starch samples were compared at once. The results are shown in Table II. It will be seen that the 3 soluble starch samples and dextrin (Merck's "Blue Label") are saccharified at the same rate.

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[4] Mathews, A. P., Physiological Chemistry, 1920, 3d ed., p. 891, New York.

TABLE II.

Comparison of soluble starch Samples 1, 2, and 3 with dextrin. "Digestibility" as determined by the copper reduction method.

Starch 2%. pH 6.9. Temp. 35° C. NaCl .05 M. Phosphate .05 M.

Six experiments with different specimens of saliva.

Sample	Mg. maltose produced per ml. in 30 minutes							Variation
Exp.	a	b	c	d	e	f	av.	%
1	14.0	11.2	9.7	12.7	13.6	13.2	12.4	
2	14.3	11.8	10.8	12.8	13.4	13.2	12.7	2.5
2	11.0	11.9	10.9	12.9	11.9	10.9	11.6	
3	11.0	12.1	10.7	13.0	12.4	11.3	11.7	1.12
3	11.1	11.5	10.2	11.1	11.6	12.4	11.3	
Dextrin	11.0	11.5	10.2	11.8	11.5	11.9	11.3	0.0

Soluble starch is prepared by incipient acid hydrolysis of starch, and by other methods not in commercial use. One source of error in the older experiments with amylase determination has been the differences in chloride concentrations in different samples; another has been acidity. In modern methods, these are eliminated by the addition of relatively large amounts of chloride and buffer salts. However, as shown here, there are other errors which are apparently caused by differences in the chemical constitutions of different soluble starch samples.

As shown above, the copper reduction method yields very closely comparable results from sample to sample of soluble starch and dextrin; the enzyme determined by this method has dextrin as its substrate (possibly also soluble starch). The iodine methods give widely discrepant values. It has been pointed out (Chesley[1]) that the order of relative digestibility of 3 soluble starch samples as measured by the viscometric method is the reverse of the order as determined by Wohlgemuth's iodine method. If the viscosity of soluble starch is caused by amylopectin, as Samec[5] believes to be the case for starch, then the viscometric method measures primarily the hydrolysis of amylopectin. As for the iodine methods, that of Wohlgemuth depends upon the complete disappearance of amylose (and other substances giving a blue iodine reaction), while the achromic methods depend upon the total disappearance of all substances giving color reactions with iodine. If the concentrations of such substances vary from sample to sample of soluble starch, then different amounts of amylase will be required to hydrolyze the digest to the end-point in a given time.

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[5] Samec, M., Comprehensive Survey of Starch Chemistry, 1928, 51, New York.

From the evidence adduced in this and a previous paper (Chesley[1]), it seems probable that the disparity in "digestibility" (as given by the various methods) is caused by varying proportionate quantities of amylopectin, amylose and dextrins in the different soluble starch samples.

Summary. The achromic iodine method, with several variations, has been investigated for its validity in determining amylase.

The iodine method is not a valid quantitative procedure for the estimation of amylase. There are considerable differences in the quantities of enzyme required to digest different samples of soluble starch to the same end-point in the same time, under identical experimental conditions.

The copper reduction method is a valid quantitative method for measuring the saccharogenic power of an amylase. Dextrin and 3 samples of soluble starch were found to be saccharified at the same rate.

Apparently the disparities in digestibility which exist among different soluble starch samples are caused by varying relative amounts of amylopectin, amylose and dextrins.

7454 P

A Method for Obtaining Stable S Colonies of Human Tubercle Bacilli.

ELEANOR G. ALEXANDER. (Introduced by William H. Park.)

From New York University.

The so-called "S" colonies of the human tubercle bacillus obtained by Petroff,[1] Reed and Rice,[2] and others, as described or shown on photographic plates, although more easily emulsified in saline and of greater virulence than R colonies, appear flat, wrinkled, dull, and irregular rather than convex, glistening, round, and smooth-edged as do S colonies of bovine and avian tubercle bacilli, and the S colonies of most other bacteria which have been dissociated. The flat colonies seem to be intermediates and not true S. This paper reports a successful attempt to dissociate human tubercle bacilli completely into S colonies resembling those of the bovine and avian types, and, moreover, to develop or modify a medium favoring the continued formation of such S colonies in pure culture by means of some substance other than anti-R serum.

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[1] Petroff, S. A., and Steenken, W., Jr., *J. Exp. Med.*, 1930, **51**, 831.

[2] Reed, G. B., and Rice, C. E., *Canad. J. Res.*, 1931, **4**, 389; **5**, 111.

The Bordet-Gengou[3] medium proved not only an excellent medium for growth, but lent itself well to experimentation. Unmodified Bordet-Gengou medium was used to maintain undissociated control strains. The human strains grown on Bordet-Gengou medium all exhibited morphology characteristic of human type colonies.[3] These appeared to be much nearer the S than the R. It was found that a small optimal amount of ferric chloride, 0.0004%, representing 2.5×10^{-4} mols of iron per liter, added to Bordet-Gengou medium stimulated the dissociation of 2 human strains studied into completely smooth, convex, glistening colonies. These S colonies were produced in pure culture by one strain in the first generation, and by the other strain in the third generation on this modified medium. The fourth and fifth generations continued to produce S colonies.

Avian R bacilli were dissociated to S on modified Bordet-Gengou medium. The optimal amount of ferric chloride in this case was 0.004%, representing 2.5×10^{-3} mols of iron per liter.

The lesions produced by the convex glistening S colonies of both avian and human strains differed radically from those produced by R bacilli. They were diffuse, inflammatory, and small or microscopic, whereas those produced by R bacilli were much fewer, limited, calcified, and larger. Virulence tests indicate that the S colonies of these strains possess somewhat greater virulence than the R. Details of experimental methods used in dissociation and virulence tests will be included in a subsequent publication.

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[3] Mishulow, L., *J. Inf. Dis.*, 1932, **51**, 416.

7455 P

Developmental Morphology of Human and Avian Tubercle Bacilli on Bordet-Gengou Medium.

ELEANOR G. ALEXANDER. (Introduced by William H. Park.)

From New York University.

These observations on the developmental morphology of human and avian tubercle bacilli are in agreement with those made by Kahn[1] with H37 and a more recently isolated human strain, on Long's medium. Bordet-Gengou medium was chosen for this study, as it differs from Long's medium in not being synthetic, and is more highly enriched.

Smears were made at daily and weekly intervals from growth on Bordet-Gengou slants which were first tested for sterility by incubation at 37°C. for 24 hours. They were then seeded with a light inoculum. Prior to planting, smears were made of the inoculums. The slides were stained by a modified Ziehl-Neelsen method[2] which had been found excellent for demonstrating non-acid-fast forms of the tubercle bacillus. Each slide prior to staining was covered by a clean strip of filter paper to protect the smears as much as possible from particles of precipitated stain which might lead to inaccurate observations. The slides used were clean and free from grease. The strains employed were 2 recently isolated human strains H3 (Mishulow) and Arilotta (Alexander), and R and S strains of a dissociated avian strain obtained through Dr. Florence Sabin.

During the first week of incubation at 37°C. no growth was visible, but smears made during the first few days revealed segmenting acid-fast bacilli, acid-fast cocci and diplococci, and some non-acid-fast cocci and diplococci. Transitional diplococci were noted which appeared to consist of one acid-fast coccus and one smaller non-acid-fast coccus. After about 5 days only non-acid-fast cocci and diplococci appeared on the smears. No acid-fast bacilli were observed.

A week or 10 days after incubation, very delicately staining non-acid-fast rods appeared on the smears. Each bacillus contained one or more deeply staining non-acid-fast bodies. Some of the bacilli stained more homogeneously than others. Many were very small, and suggested non-acid-fast cocci surrounded by a mere wisp of cytoplasm.

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[1] Kahn, M. C., *Amer. Rev. Tuberc.*, 1929, **22**, 150.

[2] Alexander, E. G., *Science*, 1932, **75**, 197.

Smears taken from 2½-to-4½-weeks-old cultures showed few granule forms, many non-acid-fast bacilli and acid-fast bacilli as well. Many were rather light red in color, and in some instances contained one or more deeply stained acid-fast bodies. Occasional forms were noted which appeared to be transitional between the non-acid-fast and acid-fast stage. These transitional bacilli contained one acid-fast body and one or more non-acid-fast bodies lying within the same bacillus. Both of the transitional forms described above indicate that the granules or bodies of the tubercle bacillus are the last portions of the organism to lose acid-fastness, and the first portions to regain or reacquire acid-fast properties.

Each of the 4 strains, including both R and S forms, followed the same course of development.

7456 P

Hemorrhages in Skin Lesions After Intravenous Injection of Starch.

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From the Department of Pathology, Cornell University Medical College.

When rabbits are injected intravenously with cholera vibrios and on the following day the same material, or *B. coli* or proteus bacilli, is again injected intravenously, hemorrhages occur in the intestines at the site of the localization of cholera vibrios. This hemorrhagic phenomenon was discovered by Sanarelli.[1] Shwartzman[2] and Hanger[3] have shown that when certain species of bacteria or their products (*B. typhosus, B. lepisepticum, B. coli*, meningococci) are introduced into the skin of rabbits and one day later the same material injected intravenously, hemorrhagic necrosis occurs in the skin at the site of the first injection. The nature of this hemorrhagic reaction is still obscure. It seems reasonable to hope that the discovery of new substances capable of producing the reaction may throw light on the nature of the phenomenon. Sickles[4] observed that bacterial filtrates, of the second injection, can be replaced by a 0.1% agar suspension but not by purified agar, galactose, 10% gelatin, or India ink. Shwartzman[5] reported that mixtures of precipitinogen and serum containing homologous precipitins injected intravenously produce hemorrhage in the skin prepared by bacterial filtrates. Sickles did not express her view as to the mode of action of the agar suspension. It is possible that the action of agar is due to its colloidal character but we have found that a suspension of collodion particles, of less than 3 micra in diameter, injected intravenously did not produce hemorrhages in areas injected with bacterial filtrates.

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[1] Sanarelli, G., *Ann. Inst. Pasteur*, 1924, **38**, 11.

[2] Shwartzman, G., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 560.

[3] Hanger, F., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 775.

[4] Sickles, G., *J. Immunol.*, 1931, **20**, 169.

It is known that the intravenous injection of agar mixed with fresh guinea pig serum and kept at 37°C. for 30 minutes produces anaphylactoid symptoms in guinea pigs, but agar alone or a mixture of agar and heated guinea pig serum are entirely inactive in this respect. Since starch, like agar, produces anaphylactoid symptoms in the guinea pig we wanted to find out if starch used as a second injection causes hemorrhagic phenomenon. Starch has the advantages that it is a relatively pure substance and its action upon the vascular system, when introduced into the blood stream, has been studied by physiologists (Bayliss and others).

In one group of rabbits from 0.1 to 0.25 cc. of filtrates from *B. coli* or meningococci were injected into the skin. On the following day, the rabbits received an intravenous injection of soluble starch or cornstarch solution. In another group of rabbits, eel serum was used to prepare the skin, since in previous experiments we found that fresh eel serum is very potent in preparing the skin for the hemorrhagic reaction. Six of 7 rabbits injected with 10 cc. of a 10% starch solution reacted with hemorrhage; the incidence of hemorrhagic reactions was slightly lower when 5 cc. of a 10% or 10 cc. of a 5% solution was used. Starch did not act as a skin preparatory agent.

After the intravenous injection of soluble starch solution, many of the rabbits appeared excited, their breathing was labored, and in some of them urination and frequent defecation was observed.

The hemorrhagic reactions appeared within from 5 minutes to 2 hours after the intravenous injection of soluble starch. According to Shwartzman and others, the hemorrhagic reaction, as a rule, occurs only between 4 to 5 hours after the injection of bacterial filtrate. It has been found by various workers that about 30% of the rabbits are refractory to the hemorrhagic reaction when bacterial filtrates are used for both injections. In the rabbits injected with 10 cc. of 10% soluble starch solution, only 1 of 7 failed to react with hemorrhage. The literature of the subject shows that from 25 to 30% of rabbits die after the intravenous injection of bacterial filtrates. No deaths occurred after the intravenous injection of soluble starch.

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[5] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 193; Shwartzman, G., *J. Exp. Med.*, 1933, **57**, 859.

7457 P

Rôle of Pressor Substances in Etiology of Arterial Hypertension.

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The theory that circulating substances play an etiological rôle in arterial hypertension has been revived recently by a series of studies carried on by Bohn and his associates.[1, 2, 3] These investigators of the Volhard clinic have reported the finding of increased amounts of pressor substances in the urine and in the blood of patients with "pale hypertension" (Volhard). In view of the important implications of Bohn's conclusions, we have reinvestigated this problem.

Because of the fact that several hormones and chemical substances are present in greater concentration in the urine than in the blood, and because large amounts of urine are readily obtainable for maximum chemical concentration, we have studied particularly extracts of urine. Blood and chest, abdominal and cerebrospinal fluids were also tested, both before and after extraction. In order to ascertain the efficacy of the alcohol-acetone fractionation of pressor and depressor substances, we have tested biologically not only the alcohol but also the acetone fraction, whereas Bohn utilized only the former. Twenty-one subjects were studied, 8 of whom were normal, 7 had malignant hypertension, 2 benign hypertension, and 4 chronic glomerulonephritis with hypertension. Extraction of a number of 24-hour urine specimens on each subject was performed. A total of 120 extracts were repeatedly tested on 55 cats. Dial and amytal given intraperitoneally were used as anesthetics, but

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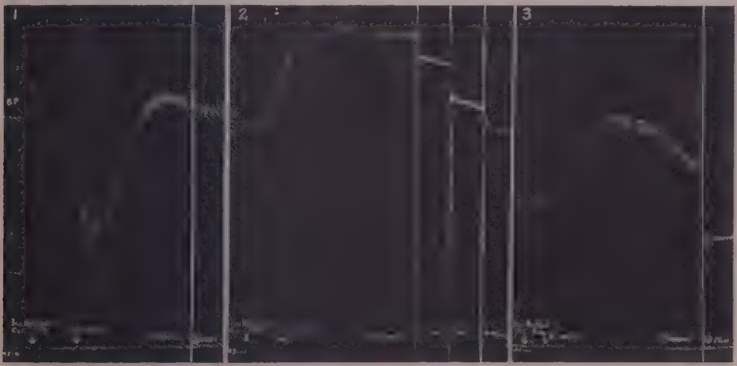
[1] Bohn, H., *Z. f. klin. Med.*, 1931, **119**, 100.

[2] Bohn, H., *Z. f. klin. Med.*, 1933, **123**, 558.

[3] Bohn, H., *Verhandl. d. deutsch. Gesellsch. f. inn. Med.*, 1933, **45**, 182.

comparative observations were also made of the effect of identical extracts on animals anesthetized with ether, chloral hydrate and urethane. Care was taken to test the same extracts in different animals, as well as to test different extracts obtained from the same subject in the same animal.

A pressor effect of over 25 mm. Hg. was obtained in the majority of subjects tested, using an amount of urine extract equivalent to one-fifth of the total 24-hour amount. The injected volume was 6 cc. Pressor effects as high as 100 mm. Hg. and over, lasting at times over 20 minutes, were obtained from both alcohol and acetone fractions. The majority of high pressor effects were obtained from the urine of normal subjects. We were unable to confirm Bohn's observation that the type of curve is as important as the height for we have repeatedly observed pure pressor curves (curve type 3 of Bohn) from the extracts of urine of normal subjects. Only rarely has this been seen in the "pale hypertension" group.



Types of blood pressure curves obtained from acetone extract fraction of urines of normal subjects.
Tracing 1. Initial pressure 200 mm. Hg; maximal pressure 220 mm. Hg (Bohn type 1).
Tracing 2. Initial pressure 192 mm. Hg; maximal pressure 280 mm. Hg (Bohn type 3).
Tracing 3. Initial pressure 126 mm. Hg; maximal pressure 200 mm. Hg (Bohn type 2).

The form, height and duration of the blood pressure curves obtained were extremely variable and were found to depend, in part, on (1) unknown variations in the subjects from day to day; (2) variations, as yet unexplained, in the response to the same extract observed both in different cats, and in the same cat at different times; and (3) the rate of injection of the extract. The type of anesthesia used has produced only quantitative changes in the reaction of the animals. No correlation was found between the responses obtained with the extracts and those obtained with epinephrin, pituitrin, saline, or by induction of the carotid sinus pressor reflex. Atropin has been found to abolish the depressor part of the curve but to have no effect upon the pressor portion. Control injections of saline at times induced a rise of as high as 30 mm. Hg for a period of one or 2 minutes' duration.

Nineteen blood extracts were made on as many patients, using both the Bohn and a modified technique. These patients included normal subjects and hypertensive subjects of the different types. Three extracts of ascitic fluid, chest fluid and spinal fluid were made, by a modified method, on 9 subjects, including both normals and hypertensives in each group of fluids. Using both the original fluid and the extract, we were unable to demonstrate any significant pressor effect. Similar results were obtained in the case of blood. Recently de Wesselow and Griffiths[4] and Page[5] were unable to demonstrate increased amounts of pressor substances in the blood of patients with arterial hypertension.

Our observations fail to confirm the claim of Bohn that increased amounts of circulating substances are responsible for "pale hypertension". The separation of pressor and depressor substances by the alcohol-acetone fractionation method proved to be impracticable.

7458 C

Effect of Heavy Water (deuterium oxide) on Viability of Mouse Sarcoma and Rat Carcinoma.

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From the Chemical and Biophysical Laboratories, Memorial Hospital, New York City.

It was clearly shown[1] that the proliferating capacity of the mouse sarcoma 180 and the mouse melanoma was unaffected by heavy water (14.8 and 40% H^2_2O) when it contained salts of Locke-Ringer solution in an isotonic amount.

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[4] de Wesselow, O. L. V. S., and Griffiths, W. J., *Brit. J. Exp. Path.*, 1934, **15**, 45.

[5] Page, I. H., Fishberg's Hypertension and Nephritis, Lea and Febiger, Philadelphia, 1934, p. 230; and personal communication.

[1] Sugiura, K., and Chesley, L. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 659.

We here present the results of experiments to determine the influence of 94% heavy water on the growth of malignant neoplasms in rats and mice. This study was made possible through the courtesy of Dr. Urey of Columbia University who supplied us with the heavy water.

The mouse sarcoma 180 and Flexner Jobling rat carcinoma were selected for the present study. The behavior of these transplantable tumors in the hosts has been reported elsewhere.

Into each of 2 weighing bottles were placed 2.5 cc. of Locke Ringer solution which was evaporated to dryness over a covered water bath. One residue was then dissolved in 2.5 cc. of ordinary distilled water and the other in 2.5 cc. of 94% heavy water, thus making isotonic solutions. The solutions were buffered to pH 7.0 approximately by adding 0.088 cc. of 0.2 M KH_2PO_4 and 0.052 cc. of 0.2 M KOH. Small pieces of tumor tissues (each weighing

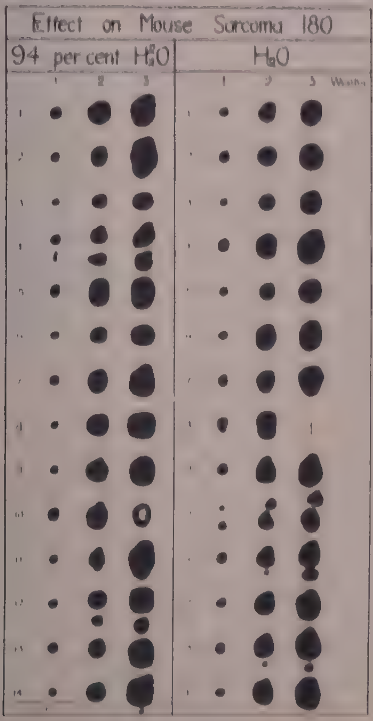

FIG. 1.

The proliferating capacity of the mouse sarcoma 180 was equally unaffected by 94% heavy water and ordinary distilled water when it contained salts of a Locke Ringer solution in an isotonic amount.

about 6 mg.) were placed in these solutions and left for 24 hours at 4-5°C. At the end of this period of time, the tumor fragments were inoculated into mice. The results are presented in Figure 1.

Each set of experiments included the inoculation of animals of about the same age with untreated tumor tissue immediately after removal from the tumor-bearing animal.

It is evident that the transplantability of mouse sarcoma 180 was not destroyed by 84% heavy water, the growths being practically the same as in the controls. Histological examinations of these treated tumor tissues showed no definite changes. It may be noted that the growth capacity of the mouse sarcoma 180 was markedly destroyed when subjected to hypotonic media for 24 hours, both in ordinary and heavy water. (Both ordinary distilled water and 40% heavy water were buffered to pH 7.0 but neither of which had been brought to isotonicity by the addition of the Locke-Ringer salts.) Fig. 2.

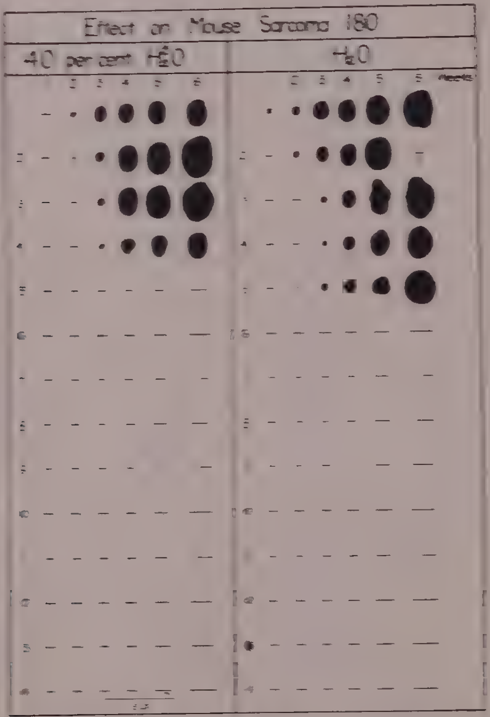

FIG. 2.

The growth capacity of the tumors was equally markedly destroyed when subjected to hypotonic media for 24 hours at 4-5°C., both in ordinary and heavy water.

It is interesting to note that an increase of the temperature to 37°C. from 4-5°C. produced a marked change in the action of both ordinary and heavy water upon the growth capacity of mouse sarcoma 180. Thus immersion of tumor fragments 6 hours or more in either 94% heavy water or ordinary distilled water, both containing salts of a Locke-Ringer solution in an isotonic amount and both adjusted colorimetrically to pH 7.0, resulted in complete inhibition of the growths.

The results of a comparative study upon the transplantable tumors indicate that the Flexner-Jobling rat carcinoma is highly sensitive to various chemical and physical agents. For this reason experiments duplicating those with mouse sarcoma 180 were made with the Flexner-Jobling rat carcinoma.

As before, fragments of fresh tumor tissue were placed in either 94% heavy water or ordinary distilled water both containing salts of a Locke-Ringer solution in an isotonic amount and both adjusted colorimetrically to pH 7.0. After standing for 24 hours at 4-5°C. the tumor fragments were implanted into young adult rats in the usual way. This study consisted of 2 groups of experiments involving a total of 72 implants. The results showed that the 94% heavy water appeared to have no inhibiting effect on the growth capacity of the Flexner-Jobling rat carcinoma. The number of tumor takes of the tumor grafts previously treated with 94% heavy water was practically the same as that obtained from ordinary water-treated tumor grafts, 67 and 75% respectively. The growth of the positive transplants in the rats in both cases was equally rapid. The microscopic examination of tumor tissues immersed in 94% heavy water for 24 hours at 4-5°C. showed a picture similar to that of the untreated tissues; the nuclei retained their staining reaction.

Summary. 1. The proliferating capacity of the mouse sarcoma 180 and the Flexner-Jobling rat carcinoma was unaffected by heavy water (94% H^2_2O) when it contained salts of a Locke-Ringer solution in an isotonic amount. 2. The growth capacity of the tumors was equally markedly destroyed when subjected to hypotonic media for 24 hours, both in ordinary and heavy water.

7459 C

The Genital-Pituitary Pathway. Non-Effect of Stimulation of Superior Cervical Sympathetic Ganglia.

H. O. HATERIUS.

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It is well known that ovulation normally occurs only as a result of coitus in the rabbit, and abundant proof exists concerning the necessity for the activity of the anterior hypophysis in this response.[1] It is well established also that the pituitary → ovary effect is a hormonal one, but it is a matter for speculation as to the nature of the genital → pituitary stimulus, *i. e.,* whether the first phase of the genital → pituitary → ovary mechanism is nervous or hormonal in nature, and, if nervous, the specific path involved. The fact that artificial insemination[2] does not induce ovulation would indicate a nervous mechanism. The problem is an attractive one, but attempts at solving it have so far been singularly unproductive, although Fee and Parkes[3] appear to have ruled out any local vaginal reflex through anesthetization of the vagina and vulva.

The meager anatomical evidence available would indicate that the nerve supply of the anterior hypophysis is derived, in part at least, through the carotid plexus. If a nervous pathway is involved in the genital → pituitary phase of postcoital ovulation, it seemed possible that a positive reaction (*i. e.,* ovulation) might be elicited through direct stimulation of the superior cervical sympathetic ganglia. On putting this to experimental test, however, uniformly negative results were obtained. In the course of the experiments,

TABLE I.
Non-effect of Stimulation of Superior Cervical Ganglia.

Animals	Anesthetic	Stimulus	Result
I-3	'Nembutal'	10 sec. at 30 sec. intervals One bilateral; 2 unilateral	Negative
II-4	Ether (light)	30 sec. at one min. intervals for 3 min. 2 bilateral; 2 unilateral	,,
III-2	,, ,,	5 sec. at 30 sec. intervals for 3 min. Bilateral	,,
IV-5	,, ,,	10 sec. in rapid succession for 1 min. 3 bilateral; 2 unilateral	,,
V-6	,, ,,	5 min. continuous stimulation Bilateral	,,

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[1] Fee, A. R., and Parkes, A. S., *J. Physiol.*, 1929, **67**, 383.

[2] Hammond, J., Reproduction in the rabbit, 1925, Edinburgh.

[3] Fee, A. R., and Parkes, A. S., *J. Physiol.*, 1930, **70**, 385.

20 rabbits (previously isolated for at least 4 weeks) were submitted to electrical stimulation. For this purpose an interrupted tetanic current was employed, using bipolar electrodes of fine Nichrome wire, insulated to the finely pointed tips and the latter spaced one millimeter apart. In most instances the strength of stimulus was adjusted to give a maximal constriction of the ear vessels. Variations in procedure are shown in Table I. In all instances exploratory operations were made 18 to 24 hours after stimulation. The animals, in addition, were re-examined 2 to 4 weeks later to determine the condition of the ovaries. One animal in each series was then sacrificed and the pituitaries were examined histologically. They did not differ in appearance from those of control animals.

In the first series 'Nembutal' (pentobarbital sodium) was used for anesthesia. Since some barbiturates apparently exercise an inhibiting effect both on the parasympathetic and the sympathetic systems,[4, 5] light ether anesthesia was used in subsequent series with, however, no difference in results.

While these experiments were in progress, Hinsey and Markee[6] reported that section of the sympathetic trunks or ablation of the superior cervical ganglia failed to prevent subsequent ovulation and pregnancy—an observation which the writer has had opportunity to confirm. Their results, as well as those reported here, seem definitely to show that the nervous pathway, if, indeed, there is a nervous pathway, is other than through the ganglia in question.

7460 C

Further Studies on Loss of Sensitivity to Anterior Pituitary-Like Hormone of Pregnancy Urine.

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From the Department of Biochemistry, McGill University, Montreal, Canada.

It is well known that both the anterior pituitary sex hormone and the anterior pituitary-like hormone of pregnancy urine (A.P.L.) lose their ovary-stimulating effect after a certain time, if given daily over a long period. Hisaw[1] has recently been able to show that this

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[4] Stavisky, G. W., *J. Pharm. and Exp. Therap.*, 1931, **43**, 499.

[5] Clark, G. A., *J. Physiol.*, 1931, **73**, 297.

[6] Hinsey, J. C., and Markee, J. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 270.

loss of sensitivity may be obtained in rabbits even by the administration of subthreshold doses of anterior pituitary sex hormone, and we[2, 3] found that the loss of sensitivity is limited to the gonadotropic preparation with which the animals have been treated previously. Animals which became insensitive to A.P.L. remained responsive to pituitary implants and vice versa.

In an attempt to determine whether this loss of sensitivity is due to the formation of specific hormone inhibiting substances as postulated by Collip[4] or simply to an insensitivity of the ovarian tissue itself, we performed the following experiments.

Five female rats, 21 days of age at the beginning of the experiment, have been injected daily with 100 R.U. of A.P.L. subcutaneously for a period of 8 months. Then they and 7 untreated females of the same size received 100 R.U. of A.P.L. intravenously. All animals were killed 1 hour after the injection. The blood of each animal was collected separately and injected in 3 doses of approximately 1.5 cc. each on 3 consecutive days into an immature rat 21 days of age, and thus tested for its A.P.L. content. The blood of all 7 previously untreated rats led to corpus luteum formation in the immature females, while the blood of the A.P.L.-insensitive animals was ineffective with one exception. The odds against obtaining such a result by chance greatly exceed 100 to 1. From this experiment we conclude that A.P.L. disappears more rapidly from the blood of the A.P.L-insensitive rat than from the blood of the normal animal.

Since this experiment does not show conclusively that the inactivation of A.P.L. has taken place in the blood itself we performed a second experiment, in which 3 units of A.P.L. were injected once a day on 3 consecutive days into each of eight 21-day-old female rats. Five of these animals received daily injections of the blood of A.P.L.-insensitive rats 3 days before and during A.P.L. administration. While all the control animals showed oestrous vaginal smears and corpus luteum formation following the injections, all the rats treated with the blood of the insensitive animals remained anoestrous and only one of them showed corpus luteum formation.

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[1] Hertz, R., and Hisaw, F. L., *Am. J. Physiol.*, 1934, **108**.

[2] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

[3] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

[4] Collip, J. B., Mount Sinai Hospital Reports, 1934 (in press).

These experiments show that the blood of animals which have become insensitive to the anterior pituitary-like substance of pregnancy urine is able to inactivate this hormone.

7461 P

Prolonged Pregnancy in Albino Rat following Injection of Pregnancy Urine Extract.

EDITH C. HOOPES. (Introduced by Franklin F. Snyder.)

From the Department of Physiology and Hygiene, Goucher College, Baltimore.

Previous observers have found marked alterations in the termination of pregnancy in the rat, following (1) injections of extracts of the corpus luteum (Nelson, *et al.*[1]), (2) injections of extracts of the anterior pituitary (Teel,[2] Hain[3]), and implants of the anterior pituitary (Hain[3]), (3) injections of the urine of pregnant women (Levin, *et al.*,[4] Hain[3]), and (4) hypophysectomy (Pencharz and Long[5]). The results of these investigations are difficult to evaluate in view of the differences in the substances administered, in their preparation and dosage, and in the parity and stage of pregnancy of the animals when treated.

In the present experiments the attempt was made to observe the duration of pregnancy in rats injected near term with a uniform dose of urine extract. The investigation was planned to take into account the factors which were found to be involved in prolongation of pregnancy in the rabbit (Snyder[6]). A satisfactory method for the prolongation of pregnancy was found to be the subcutaneous injection of 75 R.U. of pregnancy urine (Antuitrin-S of Parke, Davis Co.) 19 days after mating. The day of recovery of spermatozoa from the vagina was designated day 1. The appearance of the placental sign, usually on the 14th day, also aided in estimating the duration of pregnancy.

In a consecutive series of 19 animals, prolongation of pregnancy occurred in 16. Usually hysterotomy was performed a few days past term. Hemorrhage or discharge from the vagina appeared in 12 cases. In one rat, laparotomy about the 40th day showed the uterus strikingly distended by fetuses so that it had the form of 3 large lumps. It was surrounded by dense adhesions to the abdominal organs.

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[1] Nelson, W. O., Pfiffner, J. J., and Haterius, H. O., *Am. J. Physiol.*, 1930, **91**, 690.

[2] Teel, H. M., *Am. J. Physiol.*, 1926, **79**, 170.

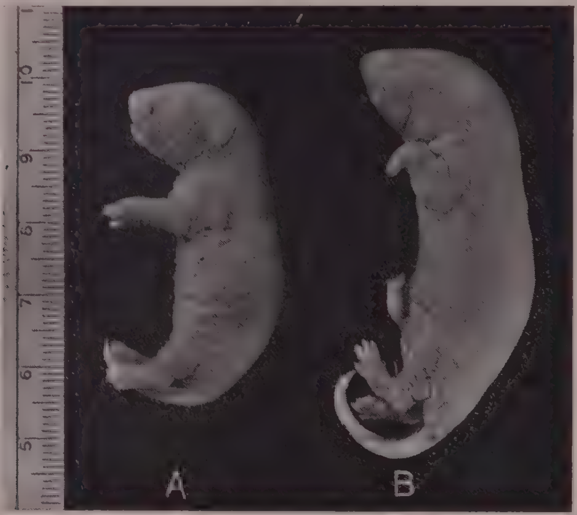
[3] Hain, A. M., *Quart. J. Exp. Physiol.*, 1932, **22**, 249.

[4] Levin, L., Katzman, P. A., and Doisy, E. A., *Endocrinology*, 1931, **15**, 207.

[5] Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

[6] Snyder, F. F., *Bull. Johns Hopkins Hospital*, 1934, **54**, 1.

Fetuses examined on the 26th day have in every case been dead. Hysterotomy on the 25th day, however, which has been performed twice, revealed living fetuses in both cases. The average length and weight of each litter was greater than normal. The uterine fluid was hemorrhagic. In another case, a litter of 4 postmature fetuses was born on the 25th day. One was living. Two of the dead fetuses measured 5.7 cm. in length and weighed over 6 gm. These data indicate that the young die between the 25th and 26th days. In one case, however, hysterotomy on the 23rd day showed a litter of 4, of which 3 were dead.



A, Normal newborn, 23rd day; B, Postmature fetus, removed by hysterotomy on the 26th day.

The photograph shows a typical postmature fetus and a control. The mothers were litter mates. Both of their litters contained 6 animals. The mother of A was untreated, and normal parturition occurred on the 23rd day. The mother of B was injected with 75 R.U. on the 19th day. On the 26th day, the 6 fetuses were removed by hysterotomy. They were all dead, and 5 of them, judging from the discoloration, must have died some time before removal. The 6th, from its color, had evidently died more recently. The average formalin length was 5.2 cm.; the average weight 8.2 gm. The control was 4.5 cm. long and weighed 6.5 gm. Other evidences of postmature development are shown by the appearance of the vibrissae, the skin, the tails, and the extremities. There was no blood in the lumen of the uterus and the placentae appeared normal.

Conclusion. Prolongation of pregnancy with development of fetuses of excessive size was regularly observed in rats following a single injection of 75 rat units of urine extract administered 4 days before term.

7462 C

Sodium, Potassium, Calcium, Magnesium and Phosphorus Content of Skeletal and Cardiac Muscle, Bladder and Uterus.

WALTER WILKINS. (Introduced by C. S. Robinson.)

From the Department of Biochemistry, Vanderbilt University Medical School, Nashville.

Few values are given in the literature for the distribution of mineral substances in the different kinds of muscle from the same animal.

Table I contains the results of the analysis of skeletal muscle (taken from the shoulder), right and left ventricle, uterus and bladder of 2 beef animals. The analyses were made by the methods de-

TABLE I.

	Mg. per 100 gm.				Milli-equivalents per kilo					mg. per 100 gm.	mMols per kilo
Tissue	Na	K	Ca	Mg	Na	K	Ca	Mg	Sum of indiv. bases	Total P	P
Beef No. 1											
Sk. Mus.	42	330	2.7	19.1	18.3	84.4	1.35	15.7	120	224	72.2
R. V.	88	248	4.3	15.9	38.2	63.4	2.15	13.1	117	238	76.8
L. V.	79	283	3.8	14.7	34.4	72.4	1.90	12.1	121	239	77.1
Uterus	219	114	10.7	9.1	95.3	29.1	5.35	7.5	137	69	22.2
Bladder	134	304	5.1	11.9	58.2	77.7	2.55	9.8	148	107	34.6
Beef No. 2											
Sk. Mus.	55	335	3.0	16.5	23.9	85.7	1.50	13.6	125	179	57.8
R. V.	92	283	4.6	16.3	40.0	72.4	2.3	13.4	128	228	73.5
L. y.	78	292	3.7	17.4	33.9	74.7	1.8	14.3	125	238	76.8
Uterus	216	98	8.2	6.5	93.9	25.0	4.1	5.3	128	61	19.8
Bladder	133	268	6.3	11.4	57.8	68.6	3.1	9.4	139	100	32.2

scribed by Cullen and Wilkins,[1] on duplicate ashings which afforded sufficient material for duplicate determinations of most of the substances in each ash.

Of all the tissues, the uterus was uniformly the richest in sodium and poorest in potassium. The total phosphorus content of both uterus and bladder was decidedly lower than that of skeletal and heart muscle. The findings for skeletal and heart muscle, including certain small constant differences in the composition of the right and left ventricles, were in general agreement with those reported by Cullen, Wilkins, and Harrison[2] and Wilkins and Cullen[3] for human tissues. These authors made no analyses of human bladder and uterus. It is recognized that the presence of blood introduced a slight error but this probably does not invalidate the result.

While the concentrations of the individual bases in the 5 tissues show much variation the sums of the bases in milli-equivalents vary within a comparatively narrow range.

7463 C

Effect of Hydrogenated Fat on Abnormal Carbohydrate Respiratory Quotients of Rats on a Fat-Deficient Diet.

LAURENCE G. WESSON AND FLORENCE C. MURRELL. (Introduced by P. D. Lamson.)

From the Department of Pharmacology, Vanderbilt University.

One of us reported[1] that extremely small amounts of fats have an influence on metabolism greatly in excess of their effect as fat *per se* because of a possible new dietary factor present in the fats. Evans and Burr[2] announced the discovery of a syndrome characterized by subnormal growth and retardation or suppression of ovulation, which developed in rats maintained on a fat-deficient diet. Burr and Burr[3] described additional symptoms of this fat deficiency disease in rats as scaly skin, necrotic tail, and hemorrhagic kidneys, and recognized[4] the fat-contained dietary factor as linoleic acid. A cooperative investigation by one of us (W.) with Dr. Burr indicated that while linoleic acid is curative for the abnormalities described by Evans and Burr and Burr, it is not curative for the metabolic abnormality described by Wesson.[5]

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[1] Cullen and Wilkins, *J. Biol. Chem.*, 1933, **102**, 403.

[2] Cullen, Wilkins, and Harrison, *J. Biol. Chem.*, 1933, **102**, 415.

[3] Wilkins and Cullen, *J. Clin. Invest.*, 1933, **12**, 1063.

[1] Wesson, L. G., *Am. J. Physiol.*, 1927, **81**, 513; *J. Biol. Chem.*, 1927, **73**, 507.

[2] Evans, H. M., and Burr, G. O., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 390.

[3] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[4] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

In the present paper, a comparison is made of the effects of an active natural fat containing linoleic acid on this abnormal metabolism before and after hydrogenation. If hydrogenation does not modify the activity of this fat, a further indication is thereby obtained that linoleic acid is not the active constituent of the fat with respect to carbohydrate metabolism.

The symptom of a deranged metabolism that appears in rats maintained on a diet deficient in fat consists of an abnormal tendency to form fat from carbohydrate, which may be alleviated by small amounts of an active fat.[5] This abnormal tendency to form fat from carbohydrate is detected by means of respiratory quotient determinations obtained during the 6 hours following the feeding of a definite amount of carbohydrate. The normal tendency to form fat from carbohydrate due to over-filling of the glycogen stores is avoided by a preliminary 18-20 hour fast. Reference is made to preceding papers[6, 7] for details of the fat-deficient diet, procedure, and previous findings.

The alcohol-soluble fraction of lard[5] was the fat used in the present work. The iodine number (Wijs) of this fat was lowered by hydrogenation[8] from 89 to 31, and a sample after saponification gave only traces of a petroleum ether-insoluble bromide (0.03%), indicating that most of the linoleic acid had become at least partially hydrogenated. The fat before hydrogenation was liquid at room temperature; after hydrogenation it required a temperature of 50-55°C. to soften and melt.

In order to avoid the effect of small amounts of possibly unchanged active substances in the hydrogenated fat, it was fed, in addition to the fat-deficient diet, to a group of 9 abnormal rats at a level that had been found to be a moderate one for this fat before hydrogenation, that is, 0.14 gm. per 100 gm. body weight daily for 2 weeks.[5] Another group of 7 abnormal rats received the same amount of the original alcohol-soluble fraction of lard, and still another group of 6 abnormal rats was given the same amount of the fat after it had been aerated at 100°C.[9]

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[5] Wesson, L. G., and Burr, G. O., *J. Biol. Chem.*, 1931, **91**, 525.

[6] Wesson, L. G., and Murrell, F. C., *J. Biol. Chem*, 1933, **100**, cii; 1933, **102**, 303.

[7] Wesson, L. G., *J. Biol. Chem.*, 1933, **100**, 365.

[8] Skita, A., and Meyer, W. A., *Ber. chem. Ges.*, 1912, **45**, 3579; Voorhees, V., and Adams, R., *J. Am. Chem. Soc.*, 1922, **44**, 1397.

The abnormal rats, before the addition of fat to their diet, gave R. Q. curves the average area of which above the line R. Q. = 1.00 was 1.31 sq. in. (1 in. = 1 hour and 0.1 unit R. Q.). After the addition of unhydrogenated fat to the diet, a group of these rats gave R. Q. curves, the average area (A, Fig. 1) of which above the line R. Q. = 1.00 was 0.20 sq. in., while the corresponding area (B, Fig. 1) given by another group of rats that received the hydrogenated fat was 0.12 sq. in. In marked contrast to these are the R. Q. curves given by the 3rd group of abnormal rats after

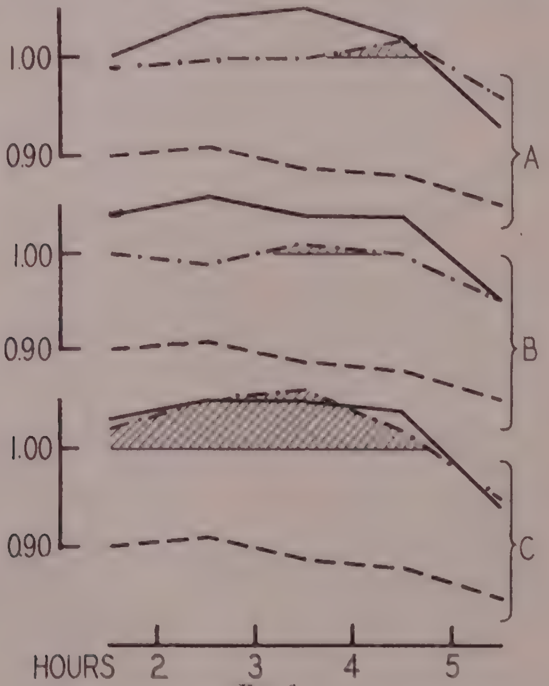

FIG. 1.

R.Q.'s following the feeding of dextrin. Solid lines represent the average R.Q. curves of the abnormal rats before the addition of fat to their diet; dots and dashes, after the addition of small amounts of fat to the diet; and dashes, the corresponding curve given by rats fed a normal diet. The cross-hatching represents the areas of definite fat formation by the abnormal rats after the inclusion of the fats in their diet.

In "A" the fat is the alcohol-soluble fraction of lard; in "B," the same fat after hydrogenation; and in "C," the same fat after aeration at 100°.

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[9] McCollum, E. V., Simmonds, N., and Shipley, P. G., *J. Biol. Chem.*, 1922, **53**, 293.

receiving the aerated fat for 2 weeks. The area above the 1.00 line of the average of these curves was 1.33 sq. in. (C, Fig. 1). Thus the aerated fat had little or no effect on the abnormal R. Q. curves, while the unhydrogenated and hydrogenated fat had a marked and similar effect on these curves.

Conclusion. The partial hydrogenation of the alcohol-soluble fraction of lard, leading to the complete or almost complete destruction of the linoleic acid contained therein, does not diminish the effectiveness of the fat with respect to the lowering of the abnormal respiratory quotients of rats on a fat-deficient diet. Further indication is thereby given that the carbohydrate factor postulated as present in natural fats is not linoleic acid, the lack of which in the diet of rats has been found to produce other deficiency symptoms.

7464 P

Penetration of Potassium Into Nitella.

A. G. JACQUES AND W. J. V. OSTERHOUT.

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

Experiments with *Valonia macrophysa* indicate that in the light the rate of entrance of potassium is increased when the external concentration of potassium or the external pH is raised above normal, but if they are reduced sufficiently below normal potassium leaves the cells. In the dark the cells fail to grow and potassium does not increase even when the concentration of potassium and the pH are simultaneously increased in the external solution.

These results have been explained by assuming that potassium enters the protoplasm chiefly as KOH, so that the rate of entrance depends on the difference between the ionic activity product [K] [OH] inside and outside.

Similar experiments with *Nitella flexilis* give the following results*: *(a)* The entrance of potassium is relatively rapid in both the dark and the light. *(b)* No growth occurs during the experiments either in the dark or the light, so that the rate of entrance is measured by the increase in the concentration of potassium. *(c)* The rate of increase of potassium is practically independent of the external pH over the range 5.7 to 8.5. Thus in a typical experiment lasting 8 days the potassium concentration increased 52% at pH 6, 58% at pH 7, and 55% at pH 8. *(d)* The rate of increase of potassium concentration at pH 7 depends on the external potassium concentration at lower concentrations, but this effect practically disappears at higher concentrations. Thus in a typical experiment (lasting 72 hours) when the external concentration of potassium was 0.0001 N the potassium concentration increased 38%, at 0.001 N 86%, and at 0.01 N 92%. *(e)* The internal pH as measured by the glass electrode did not change much from 5.5 during the rapid entrance of potassium. *(f)* The chloride concentration in the sap increases at about the same rate as the potassium concentration. *(g)* It was found that ultrafiltration of the sap (which removes a considerable amount of organic material not thrown out by centrifugalization) does not alter the potassium concentration, so that no appreciable amount of potassium is held in the sap by colloids.

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* The technique resembles that previously described but the analyses were carried out according to the methods of Emich.

Nitella appears to differ from *Valonia* in that (unless the pH just inside the inner protoplasmic surface of *Nitella* is greater than measurements of the sap as a whole indicate) potassium can enter when the ionic activity product [K] [OH] is greater inside than outside. This requires energy.

In both *Nitella* and *Valonia* the ionic activity product [K] [Cl] is greater inside than outside.

In these experiments the ionic activity product [K] [HCO_3] is in some cases greater outside than inside and it is possible that potassium enters in part in this form. It may also enter in other forms, *e. g.*, as KOH. If KOH reacts with a constituent HX of the protoplasm to form KX which then diffuses into the vacuole (as assumed in previous papers) it is evident that when the amount of HX is limited an increase of potassium or of pH beyond a certain point will produce very little increase in KX and consequently very little increase in the rate of entrance of potassium. Similar reasoning would apply to $KHCO_3$ if it reacted with HX but not if it passed through the protoplasm as $KHCO_3$.

7465 P

Experimental Studies on the Developing Perch (Perca flavescens Mitchill).

J. M. OPPENHEIMER. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratories, Yale University.

The records of experimental analyses of the embryogenic processes in the Teleost are comparatively meagre. The results of defect-experiments performed on Fundulus eggs by Morgan,[1] Lewis,[2, 3] and Hoadley[4] indicate that the ultimate limitation of cellular potencies occurs during gastrulation, but they supply no conclusive evidence concerning the nature of the mechanisms whereby the limitation is accomplished. The time is now ripe for experimental investigation and analysis of Teleost development in the light of the modern concepts of experimental embryology.

The eggs of the yellow perch were used for experimental material in the present research. Transplantation of the tissue corresponding to the dorsal lip of the amphibian blastopore induces the formation of a secondary embryo; the experiment and results are comparable to those of Spemann[5] on the egg of Triton.

The cytoplasm of the perch egg is large in amount in comparison with the yolk. The germ-ring forms and gastrulation commences when almost one-half the yolk is covered by the blastoderm. Early in gastrulation the lip of the blastoderm at which invagination is principally localized induces the formation of a secondary embryo when transplanted to an extra-embryonic region near the germ-ring of an egg the same age as the donor. The induced embryos are only slightly smaller than the normal, and have been obtained complete as far anterior as mesencephalon and myelencephalon. The parts formed are perfect. Active motility and vigorous heart-beat developed in the oldest induced embryo. The primary embryos are normal.

Inductions have also been attained by transplanting dorsal lip from later stages of gastrulation to eggs the same age as the donors. Embryos induced in this fashion are smaller and less complete than those induced by younger dorsal lip. This result may be due partly to the less ready incorporation of the graft into the thin membrane forming the later blastoderm than into the thicker tissue constituting the earlier. It may also indicate a change in the activity of the transplant or in the reactivity of the host.

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[1] Morgan, *J. Morph.*, 1895, **10**, 419.

[2] Lewis, *Anat. Rec.*, 1912, **6**, 1.

[3] Lewis, *Anat. Rec.*, 1912, **6**, 325.

[4] Hoadley, *J. Exp. Zool.*, 1928, **52**, 7.

[5] Spemann, *Arch. f. Entw.-mech. d. Org.*, 1918, **43**, 448.

In several experiments, cells from very young blastulae—well before gastrulation—were transplanted to blastulae of the same age, generally without visible effect. In one case, however, embryo-formation was apparently regular, but at the tailbud stage an extra row of 6 unpaired somites together with other tissue not diagnostically distinguishable by gross examination extended between the tailbud of the embryo and the yolk. As the tail of the embryo lengthened, the extra tissue was protracted also, and a third fin formed along the side of the tail, which was supplied in the normal manner with dorsal and ventral fin. More experiments are necessary to ascertain what materials other than dorsal lip can cause induction.

Occasionally the dorsal lip transplants fail to induce embryo-formation but preserve their integrity either within the normal embryo or on the yolk-sac. Some of those within the embryo produce or induce appropriate local structures; those on the yolk-sac undergo considerable self-differentiation. One graft of early dorsal lip, marooned on the yolk-sac epithelium, differentiated to form auditory vesicle and either self-differentiated or induced heart. The dorsal lip material giving rise to this graft was removed from a donor the same age as the donors of the dorsal lip transplants which induced embryos complete only as far anterior as the ear level.

The parts played by inductive processes and by self-differentiation in Teleost morphogenesis can now be studied. The dorsal lip of the Teleost gastrula as well as the corresponding region of the chick[6] and amphibian egg induces embryo-formation. The factors controlling development are similar in members of these 3 vertebrate groups.

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[6] Waddington, *Arch. f. Entw.-mech. d. Org.*, 1933, **128**, 502.

7466

Ant. Pituitary Changes in Adult Male Rats Following Thyroxin Injections or Thyroid Feeding.*

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The authors are not aware of any literature which describes cytological or even histological changes in the anterior pituitary due to thyroid feeding or injections. That such changes should occur might be anticipated from reported structural changes in the pituitary following thyroidectomy[1] and also from the few reported instances of pituitary weight changes after thyroxin administration. Even these reports are contradictory. A further indication that structural changes may occur in such pituitaries is found in the reports that hyperthyroid rats show an increased sex-stimulating potency.[2, 3] Weichert[4] demonstrated a prolonged dioestrus in rats after thyroid administrations, and in a subsequent work demonstrated prominent persistent functional corpora lutea.[5] All of these data refer to female rats. The present paper deals with cytological changes in the adult male rat and will be supplemented by a subsequent description of the female, which we now have reason to believe will show differences. The pituitaries of 36 adult albino male rats, purchased at Cambridge, England, which had been made hyperthyroid by injections of sodium thyroxin (British Drug House, London) or by feeding desiccated gland (Burroughs, Wellcome & Co. "Tabloids"), were studied. The thyroid tabloids were powdered on a glass plate and thoroughly mixed with a few cc. of thick, sweetened, condensed milk. The rats were observed until they finished eating the mixture, which they did avidly, and we believe that all of the thyroid was ingested. The manufacturers state that a tabloid has the equivalent of .324 gm. of fresh tissue. Each rat received one tabloid daily for 30 days. Those rats which were made

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council, administered by Dr. P. E. Smith.

† National Research Fellow.

[1] Trautmann, A., *Frankf. Zeit. f. Path.*, 1916, **18**, 173.

[2] Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1930, **45**, 215.

[3] Van Horn, W. M., *Endoc.*, 1933, **17**, 152.

[4] Weichert, C. K., *Physiol. Zool.*, 1930, **3**, 461.

[5] Weichert, C. K., and Boyd, R. W., *Anat. Rec.*, 1933, **58**, 55.

hyperthyroid by injection received varying doses of from .028 to .28 mg. of sodium thyroxin daily for 30 days (Smelser).

In addition to these experiments, a series of 6 rats from our Long-Evans strain were fed a total of from 1440 to 1840 mg. of dried Armour's thyroid over a period of from 16 to 23 days (Clark) and a second series of 20 rats from this strain was injected similarly to those described above (Smelser). All animals showed distinct but varying loss in body weight, and the accessory reproductive organs were atrophic or castrate in type. Some of the rats had been unilaterally castrated before thyroid administration. The pituitary changes of such rats are similar to those of treated normals. The pituitaries of the unilateral castrates, uninjected, used as controls, cannot be distinguished from the normal reference control.

The anterior pituitaries showed structural changes consistent in all hyperthyroid animals. These changes were briefly as follows:

The basophiles are of maximum size, markedly increased in number, and castration cells are numerous. Augmentation of the basophiles with few castration changes follows the administration of smaller thyroid doses.

The basophilic changes are in almost all respects similar to those of the castrate both progressively and in the final stage assumed. In the Helly-fixed pituitaries of the British series (all others fixed by the method previously described by Severinghaus[6]) the colloid-like substance of the basophilic castration cells has a tendency to clump into large irregular masses. The mitochondria seem larger and more numerous than in the previously studied cells of castrate male rats.

The acidophiles of treated animals average larger, stain more brilliantly than those of the normal, and show marked hypertrophy of the Golgi and mitochondrial components of the cytoplasm. In several instances, numerous acidophiles had deeply chromatic and pyknotic nuclei. These changes are in direct contrast to acidophilic changes in the castrate rat[7] where the acidophiles decrease in size and prominence.

The chromophobes appear reduced in amount, possibly due to the prominence of the increased basophilic and acidophilic areas. No cell counts have been made, many of the changes described above being of such order of magnitude that the pituitaries of the thyroid treated animals may be readily differentiated from the controls even by examination with low power of the microscope.

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[6] Severinghaus, A. E., *Anat. Rec.*, 1932, **53**, 1.

[7] Severinghaus, A. E., *Anat. Rec.*, 1933, **57**, 149.

7467

Ant. Pituitary Changes in the Adult Male Rat Following Thyroidectomy.*

A. E. SEVERINGHAUS, GEORGE K. SMELSER† AND HELEN M. CLARK.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

The authors, in the preceding paper, have reported changes in the anterior pituitary of the adult male rat following administration of thyroid. The present paper deals with the changes which occur after thyroidectomy.

The literature is replete with descriptions of pituitary changes following thyroidectomy. Numerous workers have reported that hypertrophy of the pituitary follows ablation of the thyroid. Simpson and Hunter[1] and Marine[2] review these findings, and Hammett[3] has confirmed them. The effect of thyroidectomy upon the gonad-stimulating potency of the pituitary has also received attention. Smith and Engle[4] and Van Horn[5] found no change in this potency although Evans and Simpson[6] reported a decrease. Histological studies of the pituitary after thyroidectomy have also been numerous. Trautmann,[7] in his admirable treatise on pituitary changes in the goat, fully reviews previous literature. Bryant[8] described in the rabbit a decrease in eosinophiles and a degenerated vacuolated hypertrophy of the chromophobes indicating a state of decreased secretion, while Kojima[9] reported that thyroidectomy, like castration, results in an increase in basophiles in the anterior pituitary of the rat, some of which are colloid filled.

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council, administered by Dr. P. E. Smith.

† National Research Fellow.

[1] Simpson, S., and Hunter, A., *Quart. J. Exp. Physiol.*, 1911, **4**, 257.

[2] Marine, D., *Physiol. Rev.*, 1922, **2**, 521.

[3] Hammett, F. S., *Am. J. Anat.*, 1923, **32**, 37.

[4] Smith, P. E., and Engle, E. T., *Anat. Rec.*, 1930, **45**, 278.

[5] Van Horn, W. M., *Anat. Rec.*, 1931, **51**, 38.

[6] Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1930, **45**, 215.

[7] Trautmann, A., *Frank. Zeit. f. Path.*, 1916, **18**, 173.

[8] Bryant, A. R., *Anat. Rec.*, 1930, **47**, 131.

[9] Kojima, M., *Quart. J. Exp. Physiol.*, 1917, **11**, 319.

The pituitaries of 9 thyroidectomized adult male rats of the Long-Evans strain were reserved for cytological study. The pituitaries were fixed, sectioned serially at 3/u, and stained as described in the preceding paper. The larynx region was removed at autopsy and carefully examined with a dissecting scope for thyroid tissue, and, although in no instance was any found, the material was preserved for serial sectioning.

Some of these rats had been unilaterally castrated prior to thyroidectomy in which case a unilateral castrate acted as control. The pituitaries of such controls are in every respect similar to those of normal unoperated controls.

Marked changes occur in the anterior lobe of the pituitary. The basophiles are increased in number, are of maximum size and give to the pituitary the castrate appearance. Many have deeply chromatic elongated nuclei. Large numbers of typical castration cells are present. This finding is unexpected after thyroidectomy, since castration cells had previously been found in the pituitaries of rats made hyperthyroid by thyroid injection or feeding. It is furthermore difficult to explain, in view of the fact that the accessory reproductive organs may be normal in weight while at the same time the pituitary shows the basophilic increase and many castration cells. The average weight of the accessories of 8 thyroidectomized rats was 2.695 gm., while that of their controls was 3.328 gm. In half of the cases, however, the accessories of the treated animals weighed more than those of their normal controls.

Although the basophiles of the thyroidectomized rats are similar to those of castrate and thyroid treated rats, the acidophiles are markedly different. Microscopic examination of serial sections reveals at once a loss of acidophilia. Closer inspection shows this to be due to degranulation of the acidophiles and to the small size of many of the cells. Certain acidophiles are greatly enlarged but show a vacuolation which closely approximates the degenerating vacuolation of the acidophiles described by Trautmann[7] in the thyroidectomized goat. Acidophilic granules in these cells are scarce, the typical Golgi apparatus is frequently fragmented, while mitochondria are numerous and often filamentous. Unusual nuclear phenomena are found, among which are nuclear inclusions similar also to those described by Trautmann, except that they take a basic stain. Large areas of chromophilic cells with Golgi of the acidophilic type are especially prominent in the anterior and posterior margins of the anterior lobe.

The advancement of any of several suggested explanations for these unusual pituitary findings is withheld pending completion of further experimental data.

7468 P

Effect of Injections of Pituitary Whole Gland Extract on Immature Alligator.

T. R. FORBES. (Introduced by R. K. Burns, Jr.)

The efficacy of the anterior hypophysis in producing precocious development of the reproductive system in lower vertebrates has been shown by the recent studies of Burns and Buyse,[1, 2, 3] and Burns,[4] who used as subjects larvae and recently metamorphosed salamanders. Reptiles have rarely figured in pituitary investigations. Herlant[5] found that injections of anterior lobe extract caused a general hypertrophy of the reproductive system of *Lacerta*. Noble and Bradley[6] state that hypophysectomy delays moulting in *Hemidactylus*. Schaefer[7] reports testicular atresia in *Thamnophilis* following hypophysectomy, with a partial restoration of the testes to the normal condition following several hypophyseal implants, and Houssay[8] produced ovulation in a single female snake, *Xenodon*, after similar implantations. All of these experiments were carried out with mature animals.

In the present work, the immature alligator, *Alligator mississippiensis*, was chosen for study. The average total length of the first group of animals used was 24 cm., and their approximate age at the beginning of the experiment was 4 months. Intraperitoneal injections into 10 animals of 0.5 cc. per animal of the Parke, Davis & Co. sheep whole gland alkaline aqueous extract were made 3 times a week, a similar group of 10 animals serving as controls. Gross examination after 6 weeks of injections, showed a great hypertrophy of the testes of the single male member of the experimental group. A slightly less, but still striking, hypertrophy of the gonads

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[1] Burns, R. K., Jr., and Buyse, A., *Anat. Rec.*, 1931, **51**, 155.

[2] Burns, R. K., Jr. and Buyse, A., *Anat. Rec.*, 1933, **58**, 37.

[3] Burns, R. K., Jr., and Buyse, A., *J. Exp. Zool.*, 1934, **67**, 115.

[4] Burns, R. K., Jr., *Anat. Rec.*, 1934, **58**, 415.

[5] Herlant, M., *Arch. de Biol.*, 1933, **44**, 347.

[6] Noble, G. K., and Bradley, H. T., *Biol. Bull.*, 1933, **64**, 289.

[7] Schaefer, W. H., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1363.

[8] Houssay, B. A., *Compt. Rend. Soc. Biol.*, 1931, **106**, 377.

of the injected females was observed, together with lengthening and increased convolution of the oviducts. Due to lack of external evidences of sex in the immature animals, the ratio of males to females in this series was purely fortuitous, but a second series (see below) showed a preponderance of male animals, thus approximately equalizing the total number of males and females in the two series.

Histological study revealed an increase of the medullary cord region of the testes of the experimental male as compared to the controls. Germ cells were present in considerable numbers. The size of the persistent mesonephroi was in inverse proportion to the gonadal hypertrophy, and the mesonephric glomeruli had almost entirely disappeared. The injected females showed similar acceleration of development, with enormous proliferation of the germinal epithelium over the surface of the ovary and a very definite change in the thickness and cytology of the oviduct mucosa. The vestigial Wolffian ducts persisted in both normal and experimental females.

In a second series of animals, averaging 48 cm. in length and approximately 11 months in age, an injection of 1.0 cc. of the same extract was given 6 times per week for 6 weeks. Nine animals (7♂:2♀) were injected, and an equal number served as controls. An even greater hypertrophy of the gonads and oviducts was obtained in the experimental animals. Histological studies of the second series showed that the vestigial Wolffian ducts in the females and Müllerian ducts in the males had disappeared, except in 2 instances in which short segments of the latter were seen in control animals. The possibility of a bisexual stage of gonad development, comparable to that reported by Risley[9] in *Sternotherus,* as well as other histological details, will be discussed at length in a later paper.

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[9] Risley, P. L., *Zeit. f. Zellforsch. u. mikr. Anat.,* 1933, **18,** 493.

7469 P

Hypophysectomy in the Pregnant Guinea-Pig.*

R. I. PENCHARZ AND W. R. LYONS.

From the Institute of Experimental Biology, University of California.

Observations regarding the effects of hypophysectomy during the course of gestation in the rat were reported.[1, 2] It was shown that the removal of the gland during the second half of pregnancy was followed by a lengthening of the gestation period by 3 to 4 days. In many instances normal young were delivered several days beyond term, and although the mammary glands were well developed, suckling was not observed. Furthermore, hypophysectomy even as early as the twelfth day of gestation had no effect upon the subsequent development of the corpora lutea of pregnancy. Such corpora lutea were in no way distinguishable from the corpora of a normal animal of the same day of pregnancy. Similar experiments have now been extended to the guinea pig and the original observation in the rat that the latter part of pregnancy is not interrupted by hypophysectomy has been confirmed.

Our findings in the rat have since been substantiated by Allen and Wiles[3] in the cat, and Selye, *et al.*,[4, 5] in the rat and mouse. The latter workers also found that milk secretion may take place for a very brief period but ceases within a few hours postpartum.

In all, 23 guinea pigs were used. Fifteen were hypophysectomized between day 34 and 36 of pregnancy, and 8 between day 40 to 41. Hypophysectomy on day 34 to 36 was invariably followed by resorption of the foetuses within 2 days after the operation. Animals subjected to the same operation day 40 to 41, delivered viable young at term. In contrast to the findings in the rat, the period of gestation was not significantly prolonged (63 to 67 days).

Three of the 8 animals which gave birth to normal young at approximately normal term, were sacrificed within 12 to 18 hours postpartum. In 2 of them a small amount of milk could be expressed from the mammary glands. From the nipple of a third pig, a watery-like secretion but no milk could be expressed. The 5 remaining mothers were allowed to remain with their litters for about 10 days, but in no case were the young suckled. The guinea pig, therefore, may show as do the rat and mouse[4, 5] a slight postpartum secretion of milk.

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* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation of New York City. These funds were administered by Dr. Herbert M. Evans.

[1] Pencharz, R. I., and Long, J. A., *Science*, 1931, **74**, 206.

[2] Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

[3] Allen, H., and Wiles, P. J., *Physiol.*, 1932, **75**, 23.

[4] Selye, H., Collip, J. P., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 589.

[5] Selye, H., Collip, J. P., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 82.

At necropsy of the 3 pigs referred to above, it was seen that the mammary glands were considerably smaller than those of a normal postpartum animal. Microscopic examination of the glands showed that the galactophore system of the hypophysectomized animals had not attained their maximum development. Small quantities of milk could be detected in the ducts, but the picture of generalized lobular and alveolar distension typical of the normal lactating animal was lacking.

A comparison of the ovaries of the hypophysectomized animals shortly after parturition with those removed from a normal postpartum guinea pig, revealed that the ovaries of the operated animals had suffered extensive regression. This was especially brought out in the condition of the corpus luteum, which had undergone marked atrophy and showed few lipoid staining granules.

Of considerable interest also was the fact that relaxation of the pubic ligaments occurred normally in the absence of a well developed corpus luteum.

7470

Response of the Left Ventricle to Changes in Output.

PAUL GREELEY AND JAY PALMER. (Introduced by D. R. Drury.)

From the Laboratory of Physiology, School of Medicine, University of Southern California.

Previous studies on the mammalian heart have demonstrated that both the diastolic and systolic volumes increase with an increase in total output, mean arterial pressure remaining constant. (Patterson, Piper and Starling,[1] Wiggers and Katz[2]). Kozawa,[3] working with the turtle's heart found that only the diastolic volume varied.

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[1] Patterson, Piper, and Starling, *J. Physiol.*, 1914, **48**, 465.

[2] Wigger, C. S., and Katz, L. N., *Am. J. Physiol.*, 1922, **58**, 439.

[3] Kozawa, S., *J. Physiol.*, 1914, **49**, 233.

The present study was made on dogs using the modified heart lung preparation of Dusser de Barenne[4] in conjunction with an apparatus designed to maintain physical conditions as constant as possible regardless of variations in output.

A cannula of large diameter was inserted into the aorta. The pulmonary artery was cannulated twice, one cannula toward the right ventricle and the other toward the lungs. The right ventricle pumped only blood flowing from the coronary system, while the left ventricle that which was allowed to return from the venous reservoir.

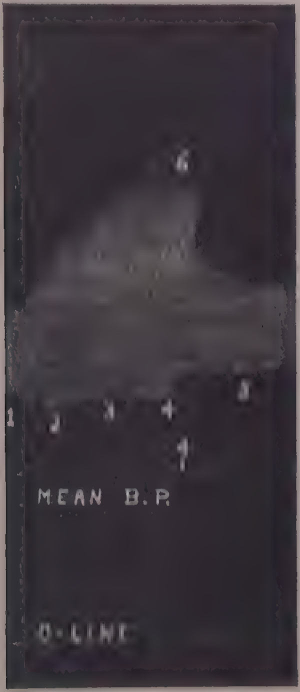

Fig. 1 shows the result obtained by rapidly increasing the input to the left ventricle. The changes in volume of the ventricles, were recorded so that systole produced a downward movement of the piston-recorder lever. Prior to (1) no blood was returning to the left ventricle from the venous reservoir, the right ventricle pumping only the coronary blood flow. At (1) blood from the venous reservoir was allowed to return to the left ventricle by way of the lungs. The diastolic volume rapidly increased to a plateau at (0). The systolic volume decreased with one exception to (2). From (3) to (4) the output of the left ventricle was about 1000 cc. per minute. The venous return to the left ventricle was blocked at (4). At (5) the volume changes of the heart were again approximately the same as at (1). Heart rate was constant and mean blood pressure increased only about one mm. Hg. during the peak of the flow, as indicated at (4).

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[4] Dusser de Barenne, *Pflüger's Arch.*, 1921, **188**, 281.

Records of increased output obtained by allowing the blood to return to the heart by way of the superior vena cava (before cannulating the pulmonary artery) showed marked increases in both the systolic and diastolic levels, the systolic rising well above that present when the heart was taking care of the coronary flow alone. Whether changes in pressure in the pulmonary artery or a combination of factors accounts for the results obtained requires further experimentation.

A paper presenting other experimental results and a detailed description of the apparatus is now in the process of preparation.

7471 P

Does Methylene Blue Form Methemoglobin?

MATILDA MOLDENHAUER BROOKS.

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These experiments were done to help clear up the present confusion as to the action of methylene blue on hemoglobin.

Barron and Harrop[1] and Warburg, Kubowitz and Christian[2] were the first to make any quantitative studies involving the interpretation of the action of methylene blue on hemoglobin. The latter state that methemoglobin does not accumulate during methylene blue catalysis when rabbit erythrocytes are placed in a saline-PO_4-glucose solution, but that it disappears as fast as formed, is reformed, disappears again and so on in a continuous cycle. The *presence of glucose* is necessary for demonstrating this catalytic effect of methylene blue because it is the reductant by which methemoglobin is transformed back to hemoglobin. Since leuco methylene blue is autoxidizable, this completes the cycle.

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[1] Barron, E. S. G., and Harrop, G. A., *J. Exp. Med.*, 1928, **48**, 207; *J. Biol. Chem.*, 1928, **79**, 65; 1929, **81**, 445; 1929, **84**, 83.

[2] Warburg, O. F., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1930, **227**, 245.

It could, therefore, be predicted that since a certain amount of glucose is present in the blood stream, methemoglobin would be reduced as fast as it was formed and that no appreciable amount of it would appear until there was no longer any glucose in the blood. This was proven by injecting more than a therapeutic dose into rats in the following experiments: rats (10 in each of 3 groups) were injected intraperitoneally with (1) $NaNO_2$; (2) methylene blue; (3) saline solution or nothing. The dose of $NaNO_2$ was 1 cc. of a 2% solution; of methylene blue, 1 cc. of a 0.1% solution, both per 100 gm. body weight. Both aqueous and saline solutions were used without any difference in results. The dose of methylene blue is 10 times that used in my former experiments[3] with CN and CO. $NaNO_2$ served as samples of blood with nearly complete transformation of hemoglobin to methemoglobin. Blood was analyzed at intervals of 15 minutes, ½ hour, 2 hours and the next day. The defibrinated blood was prepared for spectrophotometric analysis by dilution to 1% in 0.4% NH_4OH aqueous solution (Ray, Blair and Thomas.[4]) This does not change the proportion of methemoglobin to oxyhemoglobin. The extinction coefficients at various wave lengths were determined spectrophotometrically, and the ratio, R, of that at 540 mμ to that at 560 mμ was compared with those plotted by Ray, Blair and Thomas for blood containing different proportions of methemoglobin and oxyhemoglobin. It is possible by this method to detect less than 2% methemoglobin in a solution of oxyhemoglobin.

Results. In the case of $NaNO_2$ even at 15 minutes, R was 1.25, indicating about 90% methemoglobin. With methylene blue, all samples showed $R = 1.65$, the same as the controls, thus indicating negligible methemoglobin formation.

To see whether dog's blood would act differently, a 23.5 kg. dog was injected *via* the femoral artery with 16 cc. of a 1% solution of methylene blue, which is equivalent to the customary clinical dose. Before injection, $R = 1.60$; 15 minutes later, 1.63; 1 hour later, 1.62, thus again indicating negligible methemoglobin formation and practically 100% oxyhemoglobin.

The same results were obtained with intravenous injections of methylene blue into rabbits. These results show that methylene blue in the doses given, does not cause the formation of appreciable amounts of methemoglobin in the blood of rats, rabbits or dogs *in vivo*. If methemoglobin were present after the injection of methylene blue it would have been detected by these methods.

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[3] Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145; 1933, **104**, 139.

[4] Ray, G. B., Blair, H. A., and Thomas, C. I., *J. Biol. Chem.*, 1932, **98**, 63.

The diminution in O_2 capacity which has been claimed to occur under these conditions proves nothing as to the presence or absence of methemoglobin, but only that under certain specified conditions certain amounts of oxygen may be liberated. Such methods cannot therefore be used in argument against the present positive proof of the absence of methemoglobin *in vivo*. Furthermore, since glucose is essential for the cyclic reduction of methemoglobin, experiments done with washed cells, or even with blood *in vitro* where glucose is absent or strictly limited in amount, are not comparable with experiments *in vivo* where methylene blue, because of a constant supply of glucose, has full scope for its catalytic action.

This error of interpretation has been made by Haggard and Greenberg,[5] Henderson,[6] and again by Richardson,[7] who measured the O_2 capacity of blood containing methylene blue and reported the formation of 8 to 12% of methemoglobin.

The concentration of methylene blue is an important factor. Warburg uses different concentrations varying as much as from 1 to 200 times the concentration of methylene blue used in my animal experiments. Different results are obtained with these different concentrations.

Williams and Challis[8] and Geiger[9] using spectrographs were not able to demonstrate the formation of methemoglobin when methylene blue was injected into humans or animals. These methods have been criticized because of their low sensitivity, but nevertheless led to correct conclusions.

Species differences in the ease of methemoglobin formation which have been reported from time to time, are quite probably due to varying concentrations or availablity of glucose in the blood stream. *In vitro* Warburg found no difference between dog, human, rabbit, guinea pig, horse, and rat bloods.

Methylene blue does not form appreciable quantities of methemoglobin in the blood stream. The explanation of the action of methylene blue in the case of CN poisoning given by Wendel,[10] Henderson,[6] and Haggard and Greenberg,[5] *i. e.*, that the formation of methemoglobin by methylene blue and its combination with CN removes the latter from the blood stream is untenable. Furthermore, the objection of Henderson[6] that methylene blue because of methemoglobin formation cannot be used in CO poisoning is also baseless, since the catalytic action of methylene blue as a reversible oxidation-reduction dye is responsible for recovery of the animal, and not its ability to form stoichometric equivalents of methemoglobin.

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[5] Haggard, H. W., and Greenberg, L. A., *J. Am. Med. Assn.*, 1933, **100**, 2001.

[6] Henderson, Y., *Science*, 1933, **78**, 408.

[7] Richardson, E. F., *J. Am. Med. Assn.*, in press.

[8] Williams, J. R., and Challis, F. E., *J. Lab. and Clin. Med.*, 1933, **19**, 166.

[9] Geiger, J. C., *J. Am. Med. Assn.*, 1933, **101**, 269.

[10] Wendel, W. B., *J. Am. Med. Assn.*, 1933, **100**, 1054.

7472 P

Relative Growth in the Pacific Edible Crab, Cancer magister

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From the School of Biological Sciences, Stanford University.

Analyses of the form in various organisms have been presented in recent years by Huxley and his students. Certain related findings incidental to a study of the life-history and growth of the Pacific Edible Crab (*Cancer magister* Dana) are here given. The data include measurements of various linear dimensions (carapace length and width, length of chela, length of the first walking leg, length and width of the sixth and seventh abdominal segments in both the male and female) of 1804 crabs. The analysis has shown the relation of various sexual differences to the onset of sexual maturity and has suggested certain correlations between body proportions and the size attained by the species.

Treated by Huxley's method of plotting the logarithm of the part on the logarithm of the whole[1] (here usually the carapace width) in most cases one or more straight lines are obtained. The slopes of these lines are the values of k, the differential growth ratio. The nature of this constant may be seen from the following formula. If the plot may be fitted by a straight line the equation of this will be

$$\log p = \log a + k \log w$$

where p represents the part considered, w the whole, here the carapace width, and a and k are constants. Transforming, this becomes

$$p = awk$$

The values obtained in *Cancer magister* range from 0.93 to 1.61.

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[1] Huxley, Julian, Problems of relative growth, 1932.

These do not differ greatly from unity, representing isogony or growth with constant form; they should be contrasted with the values found by Shaw[2] and Huxley in *Inachus*, *Carcinus* and *Pachygrapsus* which range between 0.32 and 3.15.

The differences between the sexes in the edible crab are of 2 kinds. Differences in the primary sexual organs and in certain of the secondary sexual characters are present in the earliest post-larval stages. With the onset of sexual maturity other secondary characters change in one or the other sex producing differences, or differences already present may become accentuated.

An analysis of the relation of carapace length to carapace width may serve to illustrate the second type of change occurring with sexual maturity. Through most of the size-range the males and females do not differ significantly, the plot giving a *k* of 0.94 indicating that in both the male and female the growth in length is proceeding rather less rapidly than the width. At a width of 10 cm., however, the points representing the females begin to differ significantly from those for the males which continue with the slope mentioned above. The females now show a length greater in proportion to the width. This changed relation is very definite and in records of moulting may permit the crab to be identified as mature while the shell just quitted is clearly immature in proportions.

This evidence is corroborated, for example, by the growth in width and length of the 7th abdominal segment in females and by the difference in growth ratios between the 6th abdominal segments in males and females at this size. Independent evidence from mating and the measurement of ovigerous females indicates that these changes in form correspond to the attainment of sexual maturity.

It is a striking fact that this species, which is one of the largest of the true crabs and so numerous as to support an extensive commercial fishery from San Francisco to central Alaska, should be characterized by low degrees of heterogony or change of form with growth. Of the crustaceans studied as exhibiting marked heterogony nearly all are of small size. We may cite the fiddler crab *Uca* in which the growth of the claw shows a *k* of 1.6 (Huxley) and *Inachus* 2.6 (Shaw).

Huxley has pointed out the consequences of the increase of size in a form such as *Uca pugnax* should the chelae continue at the same ratio (about 1.6). With an increase from 3.6 to 24 gm., which is still a relatively small size for a crab, the claw would come to equal all the rest of the body.

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[2] Shaw, M. E., *Brit. J. Exp. Biol.*, 1928, **6**, 145.

The study of *Cancer magister* furnishes the opposite side of the picture, a species eminently successful as indicated by numbers and of large size (reaching a maximum of about 8½ inches and 3 lb.) in which form changes relatively slightly and slowly. That a high negative correlation exists between the degree of heterogony and the final size in a species will be clear to anyone examining an extensive collection of crustacea. Unusual and bizarre form can only result from markedly heterogonic growth and such species are almost invariably small. We have no data justifying speculation on the causal relation involved.

7473 C

Experimental Production of "Cretinism" by Thyro-Cytotoxin.

JAMES E. MORGAN AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

This work* was undertaken to ascertain if a condition simulating cretinism might be obtained by the injection of a "thyro-cytotoxic" serum into young rabbits. We were stimulated to make such a study in view of the work of Hektoen and Schulhof,[1] who were able to prepare an "antithyroglobulin", and of Portis,[2] Yates,[3] and MacCallum,[4] who studied the histology of the thyroid after injecting "thyrocytotoxic" sera.

The antigen was prepared as follows: Healthy adult rabbits were anesthetized and bled. The thyroid was then perfused *via* the carotids with normal saline solution, the thyroid being gently massaged occasionally, until the perfusate returned clear. The total time of perfusion was about 45 minutes. The thyroid was then removed, minced and ground in a mortar with prepared sand and normal saline. The suspension was permitted to stand overnight, after which it was centrifuged slowly to remove insoluble residue. The supernatant opalescent fluid was used as the antigen. Aseptic precautions were used throughout the various procedures.

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* This work was started by Dr. Paul H. Kanai (who had collaborated in a previous study with Doctors Hektoen and Dragstedt[5] and whose untimely death ended his work on an attempt to produce cretinism *in utero* by thyro-cytotoxic serum. He was successful only in showing by the precipitin reaction that the antiserum passed through the placenta of rabbits).

[1] Hektoen and Schulhof, *J. Am. Med. Assn.*, 1923, **80**, 386.

[2] Portis, *J. Inf. Dis.*, 1904, **1**, 127.

[3] Yates, *Univ. Penn. Med. Bull.*, 1903, **16**, 195.

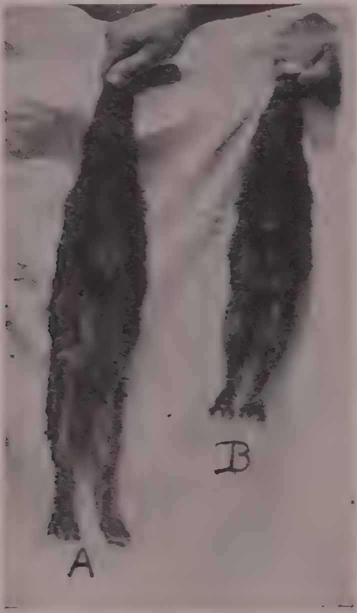
[4] MacCallum, *Med. News*, 1903, **83**, 820.

[5] Hektoen, Kanai, and Dragstedt, *J. Am. Med. Assn.*, 1925, **84**, 114.

The antigen was injected intramuscularly into healthy adult hens at 4-day intervals throughout the period of the experiment. Other hens were injected with rabbit serum, the serum of these hens serving as a "control serum". The precipitin titre was determined by the ring-test method. When the precipitin titre of the fowls reached 1 to 50,000, the serum was used for our experiments.

Litters of rabbits that numbered 7 or more were used. Injections of the "thyro-cytotoxic" serum and "anti-rabbit" serum control serum were started when the rabbits were 10 days old and were given intraperitoneally to 5 litters every fourth day and later to 3 litters every second day. Two members of the litter received the "thyro-cytotoxic" serum from the fowls treated with the thyroid, 2 received the "control anti-serum" from the fowls treated with rabbit serum, and the others were used as untreated controls.

For evidence of cretinism, the growth curve was followed, and the rate of growth of hair after shaving an area on the abdomen was observed.



A. control treated with “anti-serum”: B. “cretin” treated with “thyro-cytotoxic” serum.

Eight litters of rabbits were used. In the first 5 litters, the treated rabbits received 1 cc. of the "thyro-cytotoxic" and the "control anti-serum" sera every 4 days. After from 3 to 6 weeks it was found that the rabbits receiving the "thyro-cytotoxic" sera were growing faster than the untreated controls and than those receiving the "control anti-serum".

It was then decided to increase the dose and the frequency of injection. After some preliminary tests with larger quantities, in which 10 cc. of serum proved to be a lethal dose, we chose 3 cc. as the dose to be used in another group of young rabbits. At the first injection only ½ cc. of the "anti-sera" was given; then this dose was gradually increased to 3 cc. within 2 weeks. After this, occasionally as much as 4 cc. were given at a single dose, depending on the amount of serum obtained from the fowls at a given withdrawal of blood.

This more intensive treatment was given to 3 litters. The growth curves are shown in Fig. 1. The 6 rabbits receiving the "control anti-serum" survived throughout the period of 12 weeks. Of the 6 rabbits receiving the "thyro-cytotoxic serum", one died at 3 weeks, one at 6 weeks, 2 between 8 and 10 weeks, of respiratory infection. The remaining 2 survived the 12 week period. After withdrawal of the "thyro-cytotoxic sera" in these 2 rabbits, an increase in growth rate was evident at about 2 weeks. A marked difference in the rate of growth of the abdominal hair after shaving was observed between the rabbits treated with "thyro-cytotoxic sera" and the "treated" and "untreated" controls. The body hair of the "cretin" rabbits was lustreless and shaggy. In addition, the cretin rabbits were apathetic and their abdomens were "pot-bellied" in type. They manifested all the outward appearances of typical cretins. The thyroid glands of the rabbits in this series were examined histologically. The thyroids of the "treated" and "untreated" controls were normal. In the cretin rabbits dying prior to 12 weeks, the thyroid was so small as to be difficult to find, thus causing us to make a wide dissection of the tissue and a careful histologic examination of the mass. In one of the 4 animals, a small macroscopic gland was found which on histologic examination showed the gland to be slightly more cellular than normal. In another, no thyroid tissue was found on one side, but on the other the thyroid appeared normal histologically. In the other 2, except for the small size, the thyroid appeared normal histologically.

In the absence of metabolic studies, it cannot be stated from the evidence obtained that true cretinism was produced. The small

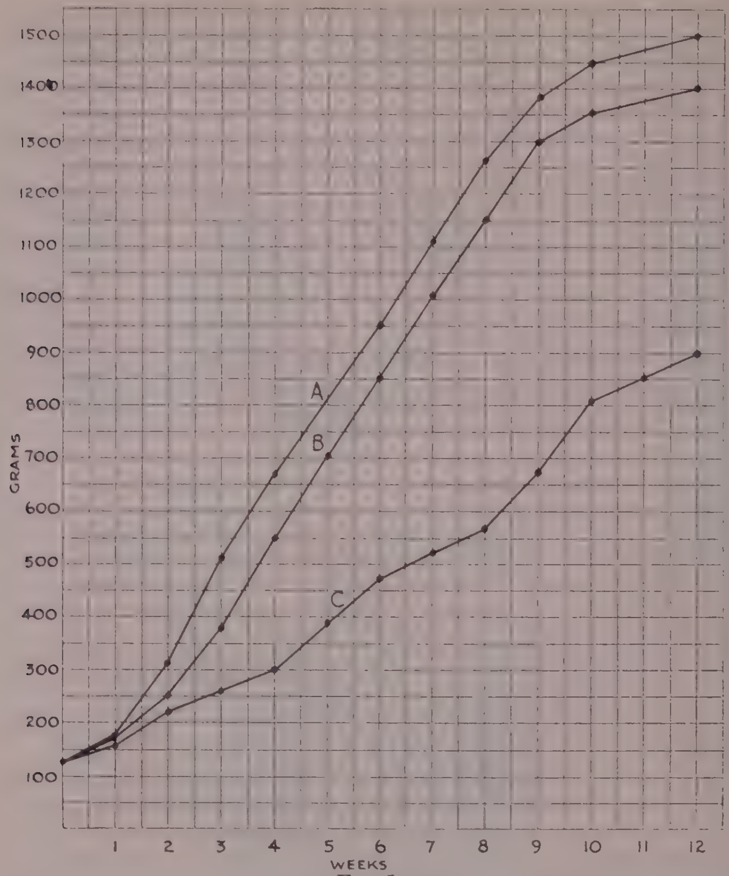


FIG. 1.

This figure shows the average growth curve of the 3 litters of rabbits referred to in the text. Curve A, the 7 untreated controls; Curve B, the 6 "treated" or "anti-serum" controls; Curve C, the 6 treated with "thyro-cytotoxic serum."

size of the thyroids of the cretin rabbits would suggest that the retarded growth of the body and of the hair was due to failure of the thyroid to develop and function normally. Before the mechanism concerned may be discussed satisfactorily, "anti-thyroglobulin" serum should be used,[6] particularly in view of Collip's observations pertaining to "anti-hormones". Since we are not in a position at this time to carry these studies further, we desire to submit the factual observations we have made.

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[6] Schulhof, *Am. J. Physiol.*, 1930, **93**, 175.

Summary. A condition simulating cretinism was produced in six young rabbits by injecting them intraperitoneally every second day with an antiserum (3 cc.) prepared by injecting hens with the proteins of the thyroid gland that are soluble in normal saline solution.

7474 P

Effect of Male Hormone Extracts, Theelin, and Theelol on the Chick Embryo.*

A. W. KOZELKA AND T. F. GALLAGHER. (Introduced by F. C. Koch.)

From Whitman Laboratory and the Department of Physiological Chemistry, The University of Chicago.

This report presents the results of a preliminary investigation. Eggs from brown and from white leghorns were injected in the albumen before or on the third day of incubation with 0.1 cc. of an ethylene glycol solution of the substance to be studied. The animals surviving on the twentieth day of incubation were killed and the tissues fixed for histological study. Comparison was made with the tissues of animals injected with the solvent alone.

The results may be summarized as follows:

A. Ten standard bird units[1] of an extract from human male urine prepared by the procedure of Gallagher and Koch[2] were injected into each egg. This preparation was contaminated with 6.3 international rat units of the estrogenic substance always found in such extracts. The results on 56 animals were: Normal males 17; normal females 21; hermaphrodites 18. This diagnosis rests on the presence of a testis and either an ovary or an ovotestis. The Wolffian ducts varied greatly in size, ranging from normal (about 0.3 mm.) to 6 mm. in diameter. No relationship was apparent between the gonad and the size of the duct. The Müllerian ducts in the females likewise exhibited great variability both between individuals and between the 2 ducts of the same animal. Eight of the 17 males showed some abnormality of the Müllerian ducts.

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* These investigations were made possible in part by a grant from the National Research Council and the Rockefeller Foundation.

[1] Gallagher, T. F., and Koch, F. C., *Proc. Amer, Soc. Biol. Chem.*, 1933, VIII, lxviii.

[2] Gallagher, T. F., and Koch, F. C., *Endocrinology*, 1934, **18**, 107.

B. Ten standard bird units of an extract of bulls' testicles prepared by the method of Gallagher and Koch[3] were injected into each egg. The estrogenic contamination in this extract was 13.3 international rat units. Results based on 51 animals showed a normal sex ratio: Normal males 25; normal females 26, and no detectable changes in the gonads. Only a slight effect was manifest on the embryonic ducts. Twenty-five animals injected with 15 standard bird units of the same extract but freed from estrogenic activity likewise resulted in a normal sex ratio: Normal males 12; normal females 13, with no differences in the gonads or ducts.

C. 100 Y crystalline theelin administered to each egg. Results on 37 animals: Males 16‡ (2 appeared abnormal); females 21 (2 appeared abnormal).

D. 100 Y crystalline theelol injected into each egg. Results based on 31 animals: Males 14† (4 abnormal and 2 doubtful); normal females 17†.

The histological changes produced by the extracts may be described as follows: The extract of bull testis produced no change. The urinary extract injections showed the cortical portion of the left gonad apparently normal ovarian tissue while the right gonad, and in many cases the medullary portion of the left, were distinctly testicular. Theelin and theelol caused a variable degree of replacement of testicular by ovarian tissue in the periphery of the left testis.

A comprehensive discussion of the results would be premature at this time. However, it is highly suggestive that the extract of bull testicles containing a higher concentration of the testicular hormone than the urinary extract gave no abnormal results. When we compare this to the relatively large number of sexually abnormal individuals obtained by treatment with urine extract, we are confronted with 2 possibilities. Either we have discovered a biological difference between the male hormone obtained from urine and that obtained from bull testicles comparable to the chemical difference discovered by Gallagher and Koch[4] or we are dealing with a new biologically active substance found in urine and not in testicles.

Further work, now in progress, may clarify the complex situation with which we are dealing.

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[3] Gallagher, T. F., and Koch, F. C., *J. Biol. Chem.*, 1929, **84**, 495.

‡ Four animals were killed on the fourteenth day.

† Two males and one female were killed on the fourteenth day.

[4] Gallagher, T. F., and Koch, F. C., *J. Biol. Chem.*, 1934, **104**, 611.

7475

Effect of Adrenalin on Insulin Sensitivity of Partially Adrenalectomized and of Hypophysectomized Dogs.

B. O. BARNES, A. S. DIX, AND J. M. ROGOFF.

From the Department of Physiology, University of Chicago, and The Laboratory of Experimental Medicine, Western Reserve University.

In a previous report[1] it was pointed out that after partial adrenalectomy in dogs, the animals became abnormally sensitive to insulin similar to that which is observed after hypophysectomy. This study was undertaken to see if adrenalin injections would delay or prevent the insulin convulsions in partially adrenalectomized and in hypophysectomized dogs. Adrenalin was injected intravenously at a constant rate for a period of 2 hours or less following a single injection of insulin intravenously. The adrenalin was diluted in sterile saline to a concentration such that one cubic centimeter per kilogram of body weight per hour would contain the desired quantity. After adrenalin was discontinued the dogs were observed for several hours for indications of hypoglycemia. Blood sugars were sometimes estimated at intervals during the experiment.

Seven dogs were used in which the dosage of insulin causing convulsions had been carefully determined. A dose of insulin was selected for each animal which consistently produced convulsions in less than 2 hours, usually within one hour. In 3 animals with both adrenals denervated and most of the medullas destroyed, this dose of insulin could be increased 2-4 times without causing convulsions during the injection of adrenalin. In one case, although the animal appeared normal when the injection of adrenalin was stopped at the end of 2 hours, convulsions occurred later. In 2 animals one adrenal had been removed and the other denervated with extensive destruction of the medulla. These animals reacted similarly to the 3 previous ones, one receiving 5 times his usual convulsive dose without showing evidence of hypoglycemia. Two animals which had been hypophysectomized several months before were quite sensitive to insulin. Injections of less than 0.5 unit per kilo regularly produced convulsions. During the administration of adrenalin these animals were able to tolerate much larger doses of insulin, although they were more likely to show convulsions when the adrenalin injection was stopped than the animals with impaired adrenal medulla.

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[1] Barnes, Scott, Ferrill and Rogoff, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 524.

A discussion of the antagonism existing between insulin and adrenalin will not be given in this preliminary report. Adrenalin dilutions were made only at the beginning of an experiment and it is not known how much deterioration occurred. However, the rate of injection at the beginning of the experiment was within the range of epinephrine secretion found by Stewart and Rogoff[2] in blood coming from the adrenal glands (0.00015-0.001 mg. per kilo per minute). More experiments are being conducted, but our results so far seem to indicate that the hypophysectomized animals require more adrenalin to prevent convulsions following insulin than animals subjected to suppression of epinephrine secretion from the adrenals. Further, it would seem probable that the hypophysectomized animal does not liberate effective amounts of adrenalin during insulin hypoglycemia.

7476 C

Studies of Renal Excretion of Creatinine. II. Volume of Distribution.

R. DOMINGUEZ.

From the Laboratories of St. Luke's Hospital, Cleveland, Ohio.

By studying the time change of the concentration of a substance in the plasma and of its simultaneous rate of excretion in the urine, the conclusion has been reached[1, 2] that both these quantities decrease exponentially to the pre-ingestion level and furthermore, that the coefficients of the time in both exponential functions are equal, on an average, for a given substance. From these 2 facts it follows that the rate of excretion of a substance obeying such laws is proportional to the plasma concentration at any time. The above statements have been verified so far only for creatinine and xylose.

In symbols we have

$$\eta = ae^{-\alpha t} \quad (1)$$

$$\xi = be^{-\beta t} \quad (2)$$

$$\alpha = \beta \quad (3)$$

$$\eta = A\xi \quad (4)$$

$$A = (a/b) \quad (5)$$

$$\eta = y - y_e \quad (6)$$

$$\xi = x - x_e \quad (7)$$

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[2] Stewart and Rogoff, *Am. J. Physiol.*, 1919, **48**, 397.

[1] Dominguez, R., and Pomerene, E., *J. Biol. Chem.*, 1934, **104**, 449.

[2] Dominguez, R., and Pomerene, E., *Proc. Am. Physiol. Soc.*, New York, March, 1934.

where y is the rate of excretion in mg. per minute, x the plasma concentration in mg. per 100 cc., y_e and x_e the mean pre-ingestion levels of y and x respectively, a, b, α, and β, constants determined from the experimental data, e the base of natural logarithms, and t the time in hours.

Equations 5, 6, and 7 are simply the definitions of A, η and ξ; Equations 1 and 2 are the empirical laws mentioned above; Equation 3 is an empirical fact, proved so far for creatinine and xylose, and Equation 4 is the result of the elimination of t between Equations 1 and 2.

A has been called the *excretion constant.*[1] It has the dimensions of flow $[l^3t^{-1}]$, and is measured in units of 100 cc. per minute. β has the dimension $[t^{-1}]$, and is, from Equation 2, the velocity constant of the disappearance of the substance from the body. It will be called, for the sake of brevity, the *velocity constant.*

When a substance is introduced into the blood stream a part passes out of the blood vessels and distributes itself in the tissue fluids. In the case of substances which are excreted (wholly or in part) this phenomenon is reversible, so that a state of equilibrium can be postulated. Under the assumption that equilibrium is maintained during the exponential decrease of excretion, the volume of body fluid dissolving the substance at the same concentration as the plasma, can be calculated. This volume will be called the *volume of distribution (V),* without any implication as to the actual distribution of the substance.

In order to include the case of substances such as xylose and creatinine, which are in part excreted by the kidney and in part disposed of in some other way, the calculation will proceed as follows:

Let us call Δz a small amount of substance excreted by the kidney in the time Δt, and Δu a small amount of the same substance removed from the body in the same time by any means consistent with the developments that follow. Then, evidently,

$$\Delta z + \Delta u = -\frac{V}{100}\frac{d\xi}{dt}\Delta t \tag{8}$$

where V is the volume of distribution and ξ is, as before, the concentration of the substance. The minus sign is due to the fact that as time elapses the concentration diminishes. If the time Δt is assumed sufficiently small, Equation 8 can be written

$$\frac{dz}{dt} + \frac{du}{dt} = -\frac{V}{100}\frac{d\xi}{dt} \tag{9}$$

Here, *(dz/dt)* is equal to the former η. It is, therefore, proportional to ξ (Equation 4). If we assume that *(du/dt)* is also proportional to ξ,

$$\frac{du}{dt} = B\xi \tag{10}$$

where the proportionality factor B may be called *utilization constant* for lack of a better name, we have, by substituting in (9),

$$A\xi + B\xi = -\frac{V}{100}\frac{d\xi}{dt} \tag{11}$$

The integration of this equation gives

$$\xi = ce^{-\frac{100(A+B)}{V}t} \tag{12}$$

where c is the constant of integration.

This equation is of the same form as the empirical result symbolized in (2).

Comparison of the coefficients of t in (2) and (12) gives

$$V = 6000(A + B)/\beta \tag{13}$$

the factor 60 arising from the fact that the rate of excretion per unit concentration *(A)* has been measured in minutes while the rate of change in concentration per unit concentration (β) has been determined in hours. With a suitable change in units the factor 6000 may be made to disappear. Equation 13 then signifies: *The volume of distribution of a substance is proportional to the sum of its rate of excretion plus its rate of utilization (both per unit concentration), and inversely proportional to the velocity constant of its elimination from the body.* Since, for any given subject, A, B, and β depend on the substance used, it is clearly worthless to speculate on the distribution of a substance in the body simply from a knowledge of the plasma concentration and an assumed percentage of body fluids.

Since A and B have the dimensions $[l^3t^{-1}]$ and β has the dimension $[t^{-1}]$, the right hand side of Equation 13 has the dimension $[l^3]$, as it should.

In view of the importance of the relation symbolized by Equation 13, in connection with the physiology of excretion and absorption, it should be pointed out that the hypothesis leading to this result, namely, that the utilization (or disposal) of the portion of the substance not excreted by the kidney proceeds at a rate proportional to the plasma concentration, is not only consistent with experiment, as already shown, but is the only hypothesis consistent with it. This can be proved as follows:

Let the rate of utilization be any function of ξ, say, $f(\xi)$

$$\frac{du}{dt} = f(\xi) \tag{14}$$

Then Equation 9, with the help of (4), becomes

$$A\xi + f(\xi) = -\frac{V}{100}\frac{d\xi}{dt} \tag{15}$$

But, from experiment, (2) holds, which upon differentiation with respect to t, yields,

$$\frac{d\xi}{dt} = -\beta\xi/60 \tag{16}$$

Substituting this expression for $(d\xi/dt)$ in (15) and rearranging, we obtain

$$f(\xi) = [(V\beta/6000) - A]\xi \tag{17}$$

that is, since the expression within brackets is constant for a given substance, the rate of utilization must be proportional to the plasma concentration in order to satisfy the experimental result represented by (2), which was to be proved. Substituting in (17) the value of V from (13), we see that the factor of proportionality is precisely B.

From (13) it follows that, in the case of a substance which is not destroyed in the body, $(B = 0)$, the volume of distribution becomes simply

$$V = (6000A)/\beta \tag{18}$$

provided—and this is essential—that the substance is *solely and entirely* eliminated by the kidneys. This provision is necessary because in the derivation of V nothing was assumed concerning any of the various means of disposal, one of which may be excretion through another emunctory. In this case, although the substance would not be utilized in the body, Equation 18 would not apply, because B, with the meaning of another excretion constant, would not be zero.

We shall show in the following paper how the utilization constant B can be calculated.

7477 C

Studies of Renal Excretion of Creatinine. III. Utilization Constant.*

R. DOMINGUEZ.

From the Laboratories of St. Luke's Hospital, Cleveland, Ohio.

In the preceding paper it was shown that, when a substance disappears exponentially from the body, and the rate of excretion by the kidneys is proportional to the plasma concentration, the rate of utilization of the portion not excreted is also proportional to the plasma concentration. The latter factor of proportionality was called utilization constant (B), in contradistinction to the former, excretion constant (A). The constant A for creatinine and xylose on one human subject has been published elsewhere. The calculation of B is as follows:

Let us call ξ_o the initial concentration at the time zero. Then the quantity initially present, M let us say, is

$$M = V\xi_o/100 = 60(A + B)\xi_o/\beta \qquad (19)^*$$

The amount excreted in the time t is, from (1),

$$\int_0^t \eta . dt = (60/\alpha)(\eta_o - \eta_t) \qquad (20)$$

the subscripts o and t meaning as usual the value of the variable at zero time and time t respectively.

The latter equation, written in terms of ξ, becomes,

$$\int_0^t \eta . dt = (60A/\alpha)(\xi_o - \xi_t) \qquad (21)$$

Therefore, since $\alpha = \beta$,

$$M - \int_0^t \eta . dt = (60/\alpha)(B\xi_o + A\xi_t) \qquad (22)$$

Since the integral in this equation has a limit, S say,

$$\int_0^\infty \eta . dt = (60A/\alpha)\xi_o = S \qquad (23)$$

we see that

$$M - S = (60/\alpha)B\xi_o \qquad (24)$$

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* In order to save space the equations of this paper and the one preceding are numbered in sequence.

and consequently, that

$$(M - S)/S = (B/A) \tag{25}$$

or

$$(S/M) = A/(A + B) \tag{26}$$

An approximation to the ratios on the left side of (25) and (26) has usually been obtained experimentally by comparing an amount S excreted in a sufficiently long interval of time to a quantity M given orally, or to the quantity M, precisely, given intravenously. The ratio (S'/M') after oral administration would be an approximate value of (S/M) in (26) if it could be assumed that all the quantity given is absorbed. The ratio (S/M) after *intravenous* injection could also be used as an approximation if it were proved that the amount excreted before the equilibrium between plasma and tissues is established, is negligible in comparison with the total amount excreted.

Lacking definite information on these points, we shall use a few figures from the literature in order to get an idea of the order of magnitude of the volume of distribution of creatinine and xylose. It should be pointed out that the "characteristic ratio" of McCance and Madders[1] is $(M - S)/S$, where M is the quantity of pentose injected intravenously.

For creatinine, the figures for (S'/M') have rarely been higher than 0.80.[2] Our own figures for Subject E.[3] yield a mean ratio (S'/M') equal to 0.73. Inasmuch as the approximation to (S/M) after oral administration is by defect, we shall take $(S/M) = 0.80$.

For xylose, the figures are very discordant, ranging, after intravenous injection, between 0.25,[4] and 0.62.[5] The mean of McCance and Madders' experiments is 0.41. We shall use this figure.

From (26), taking the above values of (S/M) and the values of A previously given for Subject E.,[3, 6] namely, 1.82 for creatinine and 0.77 for xylose, we find that the utilization constant, B, of creatinine is equal to 0.46 and that of xylose 1.06, which enables us to calculate the corresponding volumes of distribution in Equation 13 of the preceding paper.

The volume of distribution of creatinine and xylose. With the assumed values of B just given, the volume of distribution, in Subject E, with a body weight of 45 kg., becomes for creatinine,

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[1] McCance, R., and Madders, K., *Biochem. J.*, 1930, **24**, 795.

[2] For literature see: Hunter, A., *Physiol. Rev.*, 1922, **2**, 586.

[3] Dominguez, R., and Pomerene, E., *J. Biol. Chem.*, 1934, **104**, 449.

[4] Fishberg, E. H., and Friedfeld, L., *J. Clin. Invest.*, 1932, **11**, 501.

[5] Keith, N. M., Power, M. H., and Peterson, R. D., *Proc. Staff Meetings*, Mayo Clinic, 1934, January 17, 43.

[6] Dominguez, R., and Pomerene, E., *Proc. Am. Physiol. Soc.*, New York, March, 1934.

$$V = 6000(1.82 + 0.46)/0.29 = 47.2 \times 10^3 \text{ cc.},$$

and for xylose,

$$V = 6000(0.77 + 1.06)/0.5756 = 19.1 \times 10^3 \text{ cc.}$$

Seeing that the volume of distribution of creatinine, if considered as water, is equivalent to the weight of the whole body, it is at once evident that there must be condensation of creatinine in the tissues, whether creatinine is uniformly distributed or not.

The large volume of distribution of creatinine clears up some of the difficulties of Heesch and Tscherning,[7] who, after stating that the excretion of creatinine is less dependent on renal activity than the excretion of phenolsulphonphthalein, say, "Vielmehr spielen die Gewebe hier die fast Ausschlaggebende Rolle; die Rückwanderung aus ihnen bestimmt das Tempo der Ausscheidung, ohne das Blut and Niere erkennbar an der Ausscheidung Anteil nehmen." If we should inject 500 mg. of creatinine intravenously in Subject E, and assume equilibrium to be reached so soon after injection that the amount excreted during this time can be neglected in comparison with the whole amount excreted, the initial concentration at equilibrium would be 500/376 = 1.33 mg. per 100 cc., and the concentration at the end of an hour (the duration of the interval in Heesch and Tscherning's experiments), 0.995 (from Equation 2 and $\alpha = 0.29$). The amount excreted in an hour would be, from (21), 126 mg. plus the endogenous amount (60×0.724), namely, 169 mg. of creatinine, a quantity which corresponds to a fall in plasma concentration from 2.37 (1.33 plus the endogenous blank, 1.04) to 2.04 (0.995 plus the endogenous blank). That is to say, on account of the small quantity injected and the large volume of distribution of creatinine, the changes in concentration are not recognizable because they lie within the province of the experimental error.

Whether the rate of the fall of plasma concentration is due mostly to the release of creatinine from the tissues and to a lesser degree to the excretory ability of the kidney, the experiments of Heesch and Tscherning are insufficient to determine. Yet, if creatinine is so readily taken up by the tissues and the process is reversible, there is no *a priori* reason why the tissues should not give it off just as readily, in which case the *tempo* of the excretion will be determined by the excretory activity of the kidneys, the tissues acting only as a reservoir so to speak.

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[7] Heesch, O., and Tscherning, R., *Z. f. Klin. Med.*, 1920, **104**, 277.

From (21) we see also that when the plasma concentration of creatinine is 20 mg. per 100 cc. the amount of creatinine in Subject E is at least (B assumed equal to zero) $(60 \times 1.82 \times 20)/0.29 = 7531$ mg., while with the same plasma concentration the amount of xylose in the body would be (B assumed equal to 0.46) $(60 \times (0.77 + 0.46) \times 20)/0.5756 = 3815$ mg. Since (under the assumptions concerning the values of B) the entire quantity of creatinine is to be excreted by the kidneys, but only about 41% of the amount of xylose, the behavior of these 2 substances as illustrated elsewhere (Fig. 1)[8] becomes easily understandable.

This fact also accounts for the large value of the excretion constant of pentoses as estimated by McCance and Madders.[1] Having no way to compute the volume of distribution, these authors assumed a volume much too large (60% of the body weight instead of 42%), and as a result the excretion constant (which they called "glomerular filtrate" after Rehberg) came out about as large as that of creatinine.

Application to the excretion constants. With the help of (9) and (11), we can write

$$\frac{dz}{dt} + \frac{du}{dt} = C\xi \tag{27}$$

where C is equal to $(A + B)$. This equation may be looked upon as the completed relation connecting the concentration of the substance in the plasma with the two processes (excretion and utilization) whose simultaneous transaction leads to the eventual elimination of the substance.

Equation 27 can be written

$$\frac{d(z + u)}{dt} = C\xi \tag{28}$$

or, introducing the symbol w for the total amount disposed of,

$$\frac{dw}{dt} = C\xi \tag{29}$$

In this equation the rate of excretion and the rate of utilization are merged into one rate of disposal, and the question which immediately arises is, would C remain constant under varying states of utilization? In other words, if B can be varied, would the equation $A + B = C$ hold, with a constant value of C. If this were the case A would vary in the opposite direction, which would amount to a new definition of renal threshold. The interesting observation of Shannon, Jolliffe and Smith[9] that, in the dog, a change in the diet alters the value of A, would be easily accounted for by the above hypothesis. As a matter of fact, since the case of xylose is only a particular instance of the general utilization of sugar, we see that when the coefficient of utilization of glucose is large, as in normal people, the value of A is practically zero, but when the utilization of glucose diminishes, as in diabetes, the value of A becomes positive and quite large.

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[8] Dominguez, R., and Pomerene, E., *J. Clin. Invest.*, in press.

The excretion constant, being subordinated to the more general phenomenon of disposal, in a large sense, loses part of its significance in renal physiology in the case of substances which, like creatinine and xylose, are in part utilized in the body and in part excreted.

7478

A Case of Myeloid Leukemia Treated with Luminal and Amidopyrine.

M. C. TERRY AND A. O. SANDERS. (Introduced by E. W. Schultz.)

From the U. S. Veterans Hospital, Palo Alto, California.

On September 15, 1933, a male negro 37 years old was admitted to the medical service with the diagnosis of myeloid leukemia. His leucocyte count was 267,000 per cu. mm. and his spleen came to the level of the umbilicus.

Four treatments by splenic irradiation in doses of 145 r, beginning October 6, brought the count down to 156,000. This effect was transitory; on November 21 the count was 269,000 and there was no reduction in the size of the spleen.

From November 21 to March 7, 1934, the patient was given subcutaneous injections of his own leucocytes, each injection consisting of the cells recovered from 10 cc. of his blood taken in citrated salt solution the day before injection. This treatment, based on an encouraging report by Lindstrom[1] and previously used by one of us (M.C.T.) with apparent benefit in 2 unpublished cases, was without avail in the case here reported, the count continuing to rise until, on March 7, it was 500,000 with the spleen as large as before.

Beginning March 8 four small doses of X-ray irradiation (72 r) were given over the chest and long bones. The irradiation of March 30 was immediately preceded by the last of the leucocyte injections, the rays being directed to the injected area. Under this treatment the count declined to 160,000 but on March 31 it had risen to 294,000. The last X-ray treatment was given April 6.

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[9] Shannon, J. A., Jolliffe, N., and Smith, H., *Am. J. Physiol.*, 1932, **101**, 625.

[1] Lindstrom, G., *Arch. du mal du coeur*, 1921, **14**, 145.

At this time reports were appearing of cases of granulopenia apparently due to the use of luminal and amidopyrine and it occurred to us that this combination of drugs might be of value in the treatment of myeloid leukemia, with its enormous increase of cells of the granular type. Accordingly we gave our patient a total of 6 grains of luminal and 20 grains of amidopyrine in 4 doses from April 11 to April 13. We repeated these drugs in the same quantities beginning May 2 and again beginning May 9. Beginning May 14 we gave 6 grains of luminal and 20 grains of amidopyrine daily for 4 days.

No untoward symptoms have appeared. Clinically the patient has improved. He has gained 4 pounds in weight in spite of great reduction of the spleen which, not noticeably affected by previous therapy, was observed on April 16 to be only one-fourth its former size. There is no edema. The spleen is now barely palpable below the ribs and percussion indicates that the upper border is not above the normal position.

The leucocyte count subsequent to beginning treatment with luminal and amidopyrine has been as follows: April 13, 104,800; April 20, 188,800; April 27, 75,000; May 6, 67,600; May 14, 66,900; May 16, 64,500; May 17, 66,500. Treatment was discontinued May 17. Since that date the leucocyte count has been as follows: May 19, 80,000; May 25, 17,900; May 29, 20,200; June 2, 22,900; June 6, 23,050; June 12, 25,550.

As the count fell it was seen that the cells most affected were the myeloblasts and the myelocytes the percentage of which, in stained slides, decreased with a corresponding percentage increase of the less immature and the normal polymorphonuclear neutrophiles.

Whether luminal and amidopyrine in dosage yet to be determined may be useful in myeloid leukemia is merely suggested by this case. That they may have some effect is not unlikely, owing to their close relation to benzol.

The authors of recent articles have observed that luminal and amidopyrine and the like can hardly be the sole cause of granulopenia in the cases connected with the taking of those drugs and that an individual peculiarity must be concerned. Reports from mental hospitals where these drugs have been given for years should be of value on this point.

It may be that only in exceptional cases of myeloid leukemia would luminal and amidopyrine have the effects we have seen in our patient. On the other hand a series of cases may disclose some peculiarity of the same order in the victims of myeloid leukemia and in those apparently normal persons who are so remarkably affected by the drugs under consideration.

7479 P

Effect of Urine of Castrate Women on the Female Guinea Pig.

SAMUEL L. LEONARD.

From the Department of Biology, Union College, Schenectady, N. Y.

The failure of pregnancy urine to produce morphological changes in the ovaries of guinea pigs similar to those produced in rats has been recognized by Jares,[1] and Selye *et al.*[2] In view of the recent discovery that the hormone in the urine of castrate women is different from that in pregnancy urine when tested on hypophysectomized female rats (Leonard and Smith[3]), it seemed of interest to compare their effects on the female guinea pig.

Five guinea pigs ranging from 120 to 250 gm. in weight were injected subcutaneously for 5 to 8 days with an extract of urine of a castrate woman.* The amounts used, based on the equivalents of whole urine, varied from 150 cc. to 400 cc. For controls, either one ovary was removed before treatment was begun (3 cases) or comparisons were made with the ovaries of uninjected pigs of similar size. (2 cases.) Two other guinea pigs were treated with 100 R.U. and 500 R.U. of pregnancy urine extract. The ovaries, thyroids, adrenals and uterus were weighed at autopsy and the ovaries were sectioned for further study.

In all cases, the ovaries of the animals receiving the urine of castrate women increased in size and showed very marked follicular development. For example, in 3 young guinea pigs, the control ovaries weighed 29.6 mg., those of the animal receiving 200 cc. equivalent of urine weighed 39.6 mg., and those of the animal receiving 400 cc. equivalent weighed 80.3 mg.

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[1] Jares, *Anat. Rec.*, 1931, **49**, 185.

[2] Selye *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 780.

[3] Leonard and Smith, PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 283.

* Acknowledgment is made to Drs. E. Kellert and H. Grice of the Ellis Hospital, Schenectady, for their coöperation in securing the urine.

The uteri in all cases were markedly enlarged and the vaginas were open. In the ovaries of the largest animal, corpora lutea were found, none being present in the controls. Thyroids and adrenals were not significantly increased in size.

These results, while not based on a large number of animals, were all consistent in producing the follicular stimulating effect which is characteristic of such urines and is in marked contrast to the effects of pregnancy urine. The guinea pig, like the hypophysectomized rat, can be used qualitatively to differentiate the urinary gonadotropic hormones.

7480 P

Studies on Ovarian Inhibiting Action of Certain Pituitary Extracts.

SAMUEL L. LEONARD.*

From the Department of Biology, Union College, Schenectady, N. Y.

It has been amply demonstrated that hypophyseal extracts containing the growth principle will prevent the gonad-stimulating action of hypophyseal or pregnancy urine extracts in immature or hypophysectomized rats (Smith, Evans, Reiss and Leonard). This inhibitory action was first ascribed by Evans and his coworkers to the growth hormone but later work led them to abandon their original thesis. Investigations along similar lines also indicated that the growth hormone is not the antagonistic principle. Results obtained on the study of this reaction constitute this report.

Sexually immature female rats were given 10 R.U. of pregnancy urine extract (Antuitrin S)† subcutaneously, which approximately doubled the weights of the ovaries in 5 days. Littermates which were concurrently injected with pregnancy urine and extracts of beef and sheep pituitaries containing growth hormone (doses 1 cc. to 5 cc.) prepared after the method of Van Dyke,[1] failed to show the increase in weight of the ovaries. The repressing effect of the pituitary extracts upon the stimulated ovaries was also revealed by the infantile uteri and the closed vaginas of the young animals. For example, the average ovarian weight of 4 rats in one litter treated with Antuitrin S was 24.3 mg., the average of 4 littermates treated in the same manner plus the hypophyseal extract was 13.6 mg., a decrease of 44%. The uteri weighed 66.7% less and the vaginas of this latter group failed to open.‡ In testing the various pituitary fractions for inhibition and in each of the subsequent tests, no less than 12 animals were used, 6 receiving the pregnancy urine and 6 receiving the combined injections. An ovarian weight of 15 mg. was taken for the uninjected controls which is the average size for immature rats of similar age in the colony.

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* Most of this work was done as a National Research Fellow at the College of Physicians and Surgeons, Columbia University, 1931-1933.

† Acknowledgment is made to Parke Davis & Co., Squibb & Co., and the Wilson Laboratories for materials used in these experiments.

[1] Van Dyke, *J. Pharm. Exp. Therap.*, 1930, **40**, 413.

The inhibiting pituitary fraction was administered intraperitoneally because subcutaneous injections with pregnancy urine usually resulted in "augmentation" in the size of the ovaries due to the presence of the hypophyseal gonadotropic hormone. The gonadotropic hormones are not as effective when given intraperitoneally (unpublished data) and failed to produce the increased gonadotropic effect, presumably because of the hasty elimination resulting from this method of administration.

Of 2 samples of growth hormones from proprietary sources, both of tested potency, one failed to inhibit the action of Antuitrin S in doses up to 5 cc., and the other repressed the ovaries in doses of 1 cc. to 3 cc. The extract which was more potent for growth failed to inhibit. The ability of a pituitary fraction to repress the development of a stimulated rat ovary is apparently independent of its growth hormone content.

Further studies on the nature of the inhibiting substance revealed that it was destroyed by boiling but not by precipitation with alcohol. When adult female rats were injected intraperitoneally with the several preparations containing growth hormone, it was observed that the ovaries increased in size instead of becoming smaller. Interestingly, the preparation which gave the best inhibition produced the heaviest ovaries in the adult rats. This seems to indicate that the properties of the inhibiting substance are more like those of the gonadotropic hormones. Experiments are being pursued at present to test this assumption.

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‡ Over 150 animals were used in these experiments, using only littermates for controls.

7481 P

Effectiveness of Vitamin D in Infancy in Relation to the Vitamin Source.

P. C. JEANS AND GENEVIEVE STEARNS.

Department of Pediatrics, State University of Iowa, Iowa City.

This report concerns the preliminary findings regarding the utilization by the human infant of vitamin D from different sources. The criterion chosen was the quantity of calcium retained by well infants given the same amount of milk and the same rat unitage of vitamin D. Irradiated evaporated milk containing 50 units,* and evaporated milk containing the unsaponifiable fraction of cod liver oil (Zucker concentrate) allowing 150 units per quart of reconstituted milk respectively, were compared with evaporated milk plus vitamin D given separately as a cod liver oil containing 40 units of D per gram. The Zucker concentrate milk was mixed with plain evaporated milk, and the quantities of cod liver oil were so chosen that the vitamin D unitage of each diet was constant for any given intake of milk, and was regulated by the amount present in the irradiated milk. The youngest infants received only 22 units daily, but by 16 weeks of age, all the infants were ingesting approximately 50 units of D daily. The plan of study was to give each of the 3 experimental diets to a group of infants of the same age and milk intake; after a period of about 6 weeks, the diets were changed so that each infant received each diet in turn. At the beginning of the study, 3 infants were from 5 to 8 weeks of age; 3 from 16 to 24 weeks; and one infant a year old was given each diet in turn.

The relations observed between calcium intake and retention per kilogram body weight are shown in the chart. The source of vitamin D used in each experiment is indicated by the different symbols. The line shows the average curve of retention:intake obtained from over 200 experiments upon infants having the same age range and milk intakes, but given approximately 125 units of vitamin D daily in the form of cod liver oil. The range of results in the present series tends to show that no source of vitamin D studied differed materially from the others in its effect upon the calcium retention. The quantity of retention observed in the group as a whole averaged about 10 mg. per kg. lower for any given milk intake than the retentions observed in previous experiments with infants given larger amounts of vitamin D. No evidence of clinical or

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* The amount of vitamin D is recorded in terms of Steenbock units throughout. The unitage of all 3 sources was verified by assay.

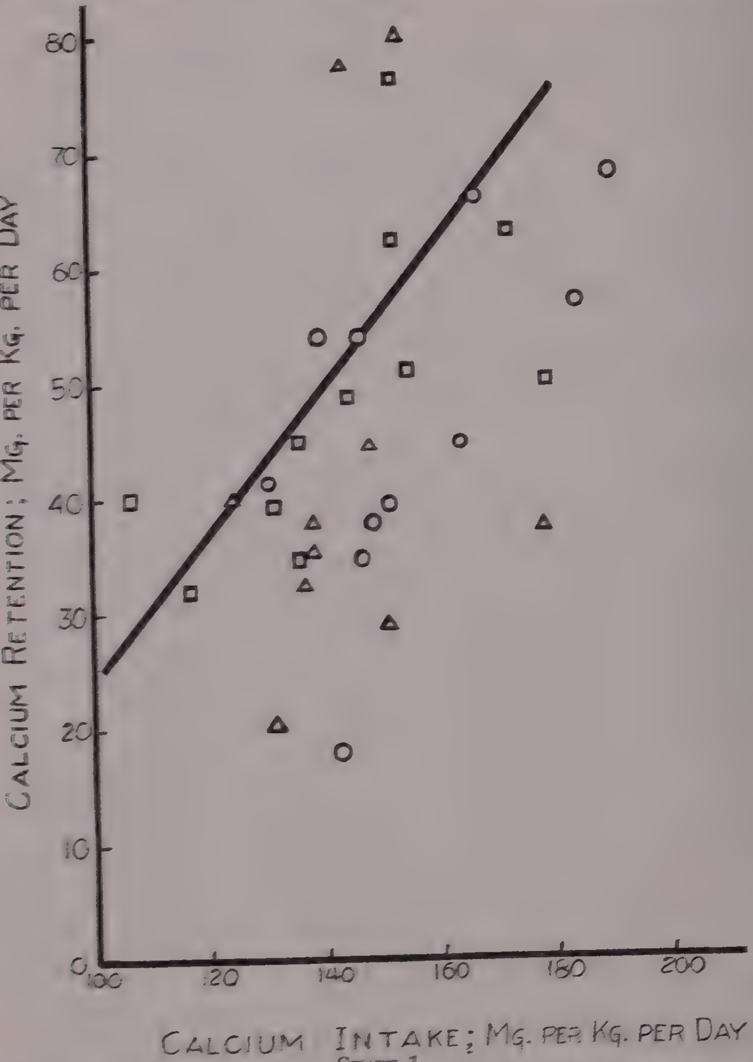

CHART 1.

Relation of intake and retention of calcium per kilogram per day of infants given from 11 to 50 rat units of vitamin D daily. Circles indicate experiments in which cod liver oil was source of vitamin D; triangles, irradiated evaporated milk; squares, cod liver oil concentrate homogenized in evaporated milk. Infants of same age were given same quantities of milk and same rat unitage of vitamin D. The line shows average curve of retention:intake of infants having same age range and milk intakes but given 135 units of vitamin D daily.

roentgenological rickets has been observed in any infant, although one baby has shown some evidence of decreasing bone density. The rate of growth in length of the group was definitely lower than the average growth of the infants given larger amounts of vitamin D.

The findings in this preliminary study indicate that for well infants, the amount of calcium retained from a given intake of milk will be determined in part by the quantity of vitamin D ingested, and that a given rat unitage of this vitamin from any one of the 3 sources studied is equivalent to the same rat unitage from either of the other 2 sources.

7482 P

Dinitrophenol in Diet, on Growth and Duration of Life of the White Rat.*

M. L. TAINTER.

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.

In connection with the present extensive clinical use of dinitrophenol (2-4 or alpha) in treating metabolic disorders or obesity, an important problem is the possible influence of the drug on the rate of growth and duration of life. Experiments on this problem have been in progress for 18 months in this laboratory and may be reported in a preliminary way now.

Three separate series of feeding experiments are being conducted, using white rats from the same strain. The rats are fed a standard adequate diet, to which are added the required amounts of alpha dinitrophenol with thorough mixing to ensure homogeneity. Food and water consumption and weights of rats are recorded at 3-day intervals. The rats are divided into groups of 5 or 6 and given the dinitrophenol in concentrations ranging from 0.0002 to 0.24%, starting shortly after weaning.

All the rats on 0.24% died before the 90th day, and 3 of those on 0.12% died by the 275th day. The lower concentrations were well tolerated as far as fatalities were concerned. Inspection of Table I shows that there was a small degree of slowing of rate of growth in the higher dosage groups. The greatest retardation at any time, with concentrations of 0.08% or less, was never more than 19%.

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* Supported in part by a grant from Rockefeller Fluid Research Fund of the School of Medicine, Stanford University.

TABLE I.
Effect of Various Concentrations of Alpha Dinitrophenol in Diet on Growth of White Rats.

Concentration of Dinitrophenol in Diet	No. Rats in Group	Aver. Wt., Gm.						
		Start	50 days	100 days	150 days	250 days	350 days	450 days
Control	5	25	166	236	268	298	296	318
.0002 %	5	24	152	222	252	280	296	312
.001 %	5	24	140	224	260	290	290	335
.005 %	5	24	136	220	255	274	281	323
Control	6	35	185	258	296	354	368	375
.001 %	6	30	175	259	292	346	380	379
.005 %	6	34	169	254	294	347	376	396
.01 %	6	33	162	250	278	294	350	357
.02 %	6	34	150	242	275	305	364	372
Control	6	33	150	207	233	273	275	
.02 %	6	36	159	226	240	285	329	
.04 %	6	34	143	197	207	255	292	
.06 %	6	33	135	191	207	232	262	
.08 %	6	31	125	176	202	239	255	
.12 %	6	35	112	167	181	217	255	
.24 %	6	32	60	all dead				

The final weights of the surviving animals do not vary significantly from the weights of the controls.

The metabolic rates of these rats, their food and water intakes, duration of life, pathologic changes in tissues, etc., will be presented in detail at the conclusion of the study.

Conclusions. Alpha dinitrophenol, added to the diet of white rats in up to maximum non-fatal concentrations, slowed down the rate of growth not more than about 19%, and did not significantly modify the body weight reached at the end of from 350 to 450 days, or in about ⅓ to ½ the span of life.

7483 P

Cultivation of Facultative Acid-Fast Bacteria from Filtrates of Leprosy.

ERNEST LINWOOD WALKER AND MARION A. SWEENEY.

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, California.

Bacteriologists and leprologists generally have regarded the facultative acid-fast organism cultivable from leprosy as a contaminant or a secondary invader of leprous lesions. Our filtration experiments with leprosy have been undertaken to exclude such adventitious organisms. Of 50 filtrates from rat leprosy through tested Seitz, Berkefeld N and W, and Chamberland L_2 and L_3 filters 16 gave positive cultures, while of 2 filtrates of human leprosy through Berkefeld N and W candles, the one through N gave a positive culture. These cultures from filtrates were in every case a pleomorphic and facultative acid-fast organism identical with the one cultivable directly from leprous lesions of the rat and man.

While it has been demonstrated by direct microscopic examination of centrifugal precipitates of filtrates that an occasional acid-fast bacillus, among the myriads present in a suspension of the organism, may pass through the pores of tested bacterial filters,[1] the probability of a chance contaminant or a microscopically unrecognizable secondary invader passing the filter in such a large proportion of the filtrates seems remote. Therefore, the repeated cultivation of this facultative acid-fast organism from filtrates of leprous tissues of the rat and man appear to furnish some support of the etiologic and endemiologic theories of leprosy advanced by us.[2]

7484 C

Chronic Toxicity of Dinitrophenol: Renal Function.*

T. L. SCHULTE and M. L. TAINTER.

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco.

In connection with the clinical use of alpha dinitrophenol (2-4),[1] it is important to know whether administration over long periods of time will produce changes in the functions of vital organs. Previous papers[2] from this laboratory contain limited data on this problem. This paper deals with the results of experiments designed to deter-

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[1] Walker, E. L., and Sweeney, M. A., *J. Infect. Dis.*, 1934, **54**, 182.

[2] Walker, E. L., *J. Prev. Med.*, 1929, **3**, 167; Walker, E. L., and Sweeney, M. A., *J. Prev. Med.*, 1929, **3**, 325.

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the School of Medicine, Stanford University.

[1] Cutting, W. C., Mehrtens, H. G., and Tainter, M. L., *J. Am. Med. Assn.*, 1933, **101**, 193; Tainter, M. L., Stockton, A. B., and Cutting, W. C., *J. Am. Med. Assn.*, 1933, **101**, 1472; Cutting, W. C., and Tainter, M. L., *J. Am. Med. Assn.*, 1933, **101**, 2099; Tainter, M. L., and Wood, D. A., *J. Am. Med. Assn.*, 1934, **102**, 1147.

[2] Tainter, M. L., Boyes, J. H., and DeEds, F., *Arch. Internat. de Pharm. et de Therap.*, 1933, **45**, 235; Tainter, M. L., and Cutting, W. C., *J. Pharm. Exp.*

mine whether dinitrophenol in repeated subfatal doses injures the kidneys.

Two series of experiments were performed, the first, a quantitative study of the urinary sediment, and the second, an estimation of the phenolsulphonphthalein excretion. Adult male rabbits were used throughout.

Urinary sediment. Six rabbits were put on a diet consisting of rolled oats and water. This diet causes an acid urine, in which the casts do not dissolve. Each sample of urine was tested with phenol red to be sure it had the required acidity. Once or twice every 24 hours the rabbits were fastened to a board, catheterized, and the bladder washed out with physiological saline solution. They were then released, and after a measured interval of several hours, catheterized again, and the urine and bladder washings quantitatively collected. The washings were centrifuged and the casts, red blood cells, white blood and epithelial cells counted, according to Addis' method.[3] From these counts, the output of the formed elements in 24 hours was readily calculated. The cast count is claimed to be the most sensitive test for renal injury, since even slight degrees of renal damage are revealed by the increased numbers of casts. The other elements are also informative, although not so significant under our conditions, since slight trauma or infection from the frequent catheterization may increase the red and white cells in the urine.

The six rabbits were observed in this way for an 11-day control period, during which time from 11 to 14 counts of the sediments were made on each one. Then 4 of the rabbits were injected subcutaneously each day with a 2% solution of alpha dinitrophenol dissolved in 1% sodium bicarbonate solution. The dosage was increased slowly until a fatal dose was reached. During the first 6 days, 6.5 mg. were injected, then 10 mg. for 4 days, 15 mg. for 3 days, 20 mg. for 3 days, and 25 mg. for 3 more days (all doses per kilo body weight). The last one of these 4 rabbits died on the third day of the 25-mg. dosage. The 2 remaining rabbits were injected with similar volumes of the bicarbonate solution as controls.

The average results for the various doses of dinitrophenol are

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Therap., 1933, **48**, 410; *ibid.*, **49**, 187; Cutting, C. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 97; Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432; Emge, L. A., Wulff, L. M. R., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 152.

[3] Addis, T., *J. Am. Med. Assn.*, 1925, **85**, 163.

TABLE I.

Average 24 hour output of formed elements in the urine of rabbits receiving dinitrophenol subcutaneously each day. Figures in parenthesis are the range of distribution.

		Dosage of Dinitrophenol				
	Control	6.5 mg.	10 mg.	15 mg.	20 mg.	25 mg.
Casts (thousands)	3.3 (0-46.7)	2.7 (0-83.3)	2.7 (0-53.3)	0 (0-0)	2.8 (0-42.3)	0 (0-0)
Red blood cells (millions)	61.2 (0-1880.0)	3.4 (0-25.7)	17.1 (0-71.0)	4.8 (0-23.6)	1.0 (0-5.8)	1.2 (0-7.2)
White blood and epithelial cells (millions)	3.2 (0-50.1)	4.9 (0-33.0)	12.4 (2.3-88.0)	5.4 (0-29.0)	3.7 (0-13.0)	11.8 (1.2-26.8)

presented in Table I. It is seen that there were no significant changes in the amount of sedimentary elements, all averages being well within the range of normal daily variations. The same was also true for each individual rabbit. The control rabbits receiving only bicarbonate solution behaved similarly. Therefore, according to this test, which is the most sensitive available for renal injury, dinitrophenol, in repeated subfatal and fatal doses, did not damage the kidneys. The kidneys of these rabbits were also examined histologically by Dr. D. A. Wood, and were found to contain no pathological alterations.

Excretion of phenolsulphonphthalein. Six rabbits were tied on boards, catheterized, and their bladders washed out with normal saline solution. Then, with the catheters still in place, 10 mg. of phenolsulphonphthalein were injected into the ear vein of each rabbit. Exactly 30 minutes later, the urine was drawn off and the bladder washed out as before. The urine and washings were combined, alkalinized, made up to a volume of 1 liter, and the dye-concentration estimated by comparing with a set of standards. This procedure was carried out 10 times on each rabbit during a 25-day control period. The average output of dye in the 30 minutes was 63%, which was taken as the average control value for these rabbits. Four of the rabbits were then injected with 20 mg. of alpha dinitrophenol per kilo subcutaneously each day for 77 days. The other 2 rabbits were injected with sodium bicarbonate solution as controls. The dye-output of each rabbit was estimated each week during the dinitrophenol period, or 11 times in all. The outputs averaged 62% during the injections, practically identical with that during the control period. It was also just as high at the end of the experimental period as it was at the beginning. Therefore, daily administration of just subfatal doses of dinitrophenol for 11 weeks failed to perceptibly diminish the excretory function of the kidney for phenolsulphonphthalein.

It is obvious that the results of these 2 series of experiments on rabbits were consistent with each other in showing that large repeated doses of dinitrophenol did not impair the functional efficiency of the kidneys.

Conclusions. 1. Daily subcutaneous injections in rabbits of alpha dinitrophenol in doses up to and including fatal ones, and for as long as 77 days, did not modify renal function as indicated by quantitative counts of the formed elements in the urinary sediment, according to Addis' method, and by excretion of phenolsulphonphthalein. 2. Therefore, it is unlikely that dinitrophenol, in ordinary clinical dosage, will injure the kidneys.

7485 C

Sex-Limitation of Cilia in Body Cavity of the Frog (R. pipiens).

J. KENNETH DONAHUE. (Introduced by W. W. Swingle.)

From the Biological Laboratory, Princeton University.

The occurrence of ciliary systems in the peritoneal lining of the body cavity of the frog has long been known (Gray[1]) but, so far as the writer is aware, the fact that they are sex-limited and appear only in the female has hitherto been unrecognized. However a comparative study of the peritoneal lining of the body cavities of 20 female and 20 male frogs (*R. pipiens*) has shown that such ciliation occurs only in the female and may be regarded as being strictly sex-limited. This study was made on frogs taken during the breeding season (April-May) at which time the ovaries of the females were greatly enlarged. Strips of the thin peritoneal lining were removed and temporarily mounted in frog Ringer for examination. The cilia show up clearly when illuminated by unfiltered light and with the diaphragm of the microscope almost completely closed. An 8 mm. objective combined with a No. 18 eyepiece proved to be the most adequate lens combination for this type of work. In the female frog ciliary systems are present throughout the ventral and lateral areas of the peritoneum, extending the entire length of the body cavity and including the pericardial region; they are particularly numerous in the peritoneal tissue immediately adjacent to the ventral abdominal vein. The ciliary beat is in an anterior direction (towards the ostia tubae). By this means the movement of the ova towards the ostia is facilitated. Since frogs kept in captivity do not ovulate, the actual transportation of the ova was not observed. In a similar study on 20 male frogs it was found that cilia were entirely absent from all parts of the peritoneal lining.

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[1] Gray, J., Ciliary Movement, 1928, Cambridge University Press, p. 163.

That the ciliation found in the peritoneal lining of the female frog might be under hormonal control seemed likely. Previously Wolf[2] showed that castration atrophy of the oviducts of the female *R. pipiens* could be prevented by daily injections (2 RU) of mammalian follicular hormone. In order to ascertain the possible relation of peritoneal ciliation to the follicular hormone a series of mature, intact male frogs were injected with 5 RU of theelin* intraperitoneally daily for varying intervals. Frog T1, examined after 9 daily injections, showed no cilia in the peritoneal lining. Frog T2 was examined after 15 daily injections and showed very small patches of active cilia in scattered regions of the peritoneal lining. Frog T3, examined 20 days after injection was initiated, revealed numerous patches of active cilia in the peritoneal lining. Frog T4, upon examination 25 days after the initial injection, showed active cilia in numerous areas of the peritoneal lining. Frog T5, examined the day following the thirtieth injection showed ciliated areas in the peritoneal lining. It should be emphasized that in the above-mentioned cases the ciliation produced by theelin did not compare with the condition found in the normal female where practically all parts of the peritoneal lining are ciliated. Doubtless a longer injection period would bring about a condition in the male frog more comparable with that seen in the female. Fifteen untreated males were examined as controls and in no instance were cilia found in the peritoneum. It appears evident from these experiments that the follicular hormone is capable of producing ciliation of the peritoneum in the male frog. It follows from this that the absence of such ciliation in the untreated male is due to a lack of follicular hormone.

The ineffectiveness of Antuitrin S in inducing ovulation in the female and amplexus in the male frog has been mentioned by Rugh.[3] In order to test its effectiveness in producing ciliation of the peritoneal lining of the male frog 4 adult males were injected with 5

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[2] Wolf, O. M., *Anat. Rec.*, 1928, **41**, 41.

* Theelin and Antuitrin S supplied through the courtesy of Dr. O. Kamm of Parke Davis & Co.

[3] Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

RU of Antuitrin S* daily for 5 days after which the dosage was increased to 10 RU daily until 25 days had elapsed. Examination of the peritoneal linings at that time showed that ciliation had not occurred in any region.

7486 C

Volume and Total Number of Glomeruli in Kidney of White Rat Estimated by Precision Methods.

ROBERT TENNANT AND WILLIAM R. THOMPSON. (Introduced by Raymond Hussey.)

From the Department of Pathology, Yale University.

The general principles of measurement of microscopic figures by projectometric methods have been treated in another communication[1] and applications to the measurement of islands of Langerhans in the pancreas have been presented.[2, 3] The greatest difficulty in this work was due to the enormous variations in their volume and shape. Since this difficulty was far less evident in the case of the glomeruli of the kidney an investigation of this field seemed indicated, and we wish to give at present some of the results so far obtained.

It has been thought preferable to make estimates free from the assumption of spherical form for glomeruli wherever possible, but to use this to furnish an independent check. However, in the material used, it was found that areas of glomerular *particles* in sections (7 micra thick) could be approximated satisfactorily by comparison of their projected images with circles of known size; and this was done rather than trace and measure them with a planimeter. A glomerulus was considered to *belong* (in the sense previously[1] defined for *islets*) to a given section if its greatest cross-sectional area lie therein. Thus it was possible from random samples and assumption of approximate spherical form to estimate directly mean volume and diameter of glomeruli in a given region. However, we are interested in data more comprehensive than this, and, if possible, free from the assumption of sphericality; *e. g.*, total glomerular tissue volume (I_s) in the sample region. Previous attempts in this direction have been based upon treatment as if the section were opaque, *i. e.*, attempting to measure all areas of glomerular (or *islet*) tissue in the first presented surface; but it is much easier in practice to focus upon the greatest cross-sectional area in the section of each glomerular particle. From the simple assumption that there may be found in serial sections only one maximal cross-sectional area for each glomerulus, it can be demonstrated that the sum of all such areas in a section except those of glomeruli actually *belonging* to it is the mean *expected* value of the sum of all glomerular area in a plane cross section (*e. g.*, the first presented surface). Furthermore, it is important to note that the expected mean number of particles in a section per glomerulus (or *islet*) belonging, M_{tn}, is one greater in the case of transparent sections than if merely the first presented surface of each is considered, *i. e.*, $M_{tn} = M_{d/t} + 1$, where d is the glomerular diameter and t is the section thickness.

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[1] Thompson, W. R., *Biometrika*, 1932, **24**, 21.

[2] Thompson, W. R., Hussey, R., *et al.*, *Biometrika*, 1932, **24**, 27.

[3] Thompson, W. R., Tennant, R., and Hussey, R., *Science*, 1933, **78**, 270.

The present experimental work has to do with measurements on the kidneys of white rats, from a colony which has been maintained for a long time in the department of Physiological Chemistry. One kidney was removed for study from each of 4 rats at an approximate age of 58 days, and the animals maintained with the object of studying similarly the condition of the other kidneys 90 days later. The volume by displacement of 5% formalin was measured; and the kidney fixed in Zenker formol solution, double imbedded and serially sectioned (at 7 micra thickness). Then by projectometric methods similar to those previously described,[3, 4] the volume of the imbedded organ was estimated and the ratio, λ^3, of this to the displacement volume of the fresh tissue found in each instance. Thus for the 4 kidneys first mentioned, $\lambda^3 = 0.547$, 0.590, 0.433 and 0.538. However, various tissue volumes compared from organ to organ showed less variation when expressed as in the *shrunken* state. Thus for these kidneys were found the following means and average deviations: for total kidney volume (T_o) 0.356 ± .014 cm³, for total glomerular volume (I_o) 7.25 ± .59 mm³, and for mean glomerulus volume (V_o) 2.90 ± .26 ($10^5\mu^3$). The estimated total number of glomeruli, N_o, in the organ was 28,400, 27,100, 25,900 and 19,800, respectively, in the 4 kidneys; mean $N_o = 25.3 \pm 2.7$ thousand. $V_o = I_o/N_o$ in the above estimates, but the mean glomerulus volume, M_{vo}, was estimated directly by means of the spherical assumption at 6 equidistant intervals in each kidney. The means of these 6 values and a.d. for each of the 4 kidneys were 2.92 ± .21, 2.91 ± .27, 2.62 ± .09, and 3.41 ± .17; and the mean of means was 2.97 with a.d. = .23 (all expressed in $10^5.\mu^3$). These values serve as a rough check on those previously given, but would be useless for this purpose were it not for the approximate stability of $M_{(v)}$ in the 6 sampled regions of each organ. However, a basis of strict comparability of the 2 methods of estimation may be had by estimating for each sample the total glomerular tissue contained (I_g), once as already mentioned, and again by the expected value of $I_g = Z_g.M_{(v)}/M_{(\eta)}$, where Z_g is the number of glomerular *particles* in the sampled region: summing each set of estimates for a given organ and obtaining the ratio of these sums. These ratios for the 4 kidneys were 1.063, 0.995, 0.980 and 1.006, respectively; and for 2 others (experimental and control) 1.032 and 0.995, respectively. This we feel indicates a far greater precision and reliability than has been obtained previously in work of this character. Neglect of the effect of focusing upon the greatest cross section of each particle in the transparent section instead of the first presented surface would have introduced an error of about 13%.

Estimates of the total number of glomeruli in the rat kidney have been made by various workers, notably by Moore,[4] whose findings agree closely with our own. However, we feel that the methods suggested above have some advantage in precision over those used previously, and in addition permit a simultaneous estimation of various volume relations.

7487 C

Antistreptolysin Content of Sera from Cases of Recurrent Tropical Lymphangitis.

P. MORALES-OTERO AND A. POMALES-LEBRÓN. (Introduced by A. M. Pappenheimer.)

From the School of Tropical Medicine, University of Puerto Rico, under the auspices of Columbia University.

Todd[1] has shown that there are in the sera of patients suffering from diseases caused by hemolytic streptococci, antibodies that neutralize *in vitro* streptococcal hemolysin. Todd[1] and Coburn[2] have demonstrated that these antibodies are found in abnormally high concentration in the sera of patients suffering from rheumatic fever and in individuals recovering from hemolytic streptococcus infections. Furthermore Coburn[2] has observed that these antibodies (antistreptolysins) are not abnormally increased in the sera of subjects with bacterial infection other than hemolytic streptococcus and he concludes that abnormally high antistreptolysin titer is strong evidence of recent infection by hemolytic streptococcus and that this relationship is apparently specific.

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[4] Moore, R. A., *J. Exp. Med.*, 1929, **50**, 709.

[1] Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

[2] Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

While studying the probable relationship of *Streptococcus hemolyticus* to recurrent tropical lymphangitis we have made antistreptolysin determinations in a group of persons suffering from this condition and in a group of apparently normal individuals. We have employed the method and culture media recommended by Hodge and Swift[3] in which the combining capacity of the streptolysin is used as an apparently more reliable index than its hemolytic power. In order to render our results comparable with those of others we are reporting them using the same system of units employed by Todd. This was made possible through the kindness of Dr. David Seegal of New York who made antistreptolysin determinations in 8 of our sera and recorded his results in units as recommended by Todd. By calculation we have been able to change our figures accordingly.

Table I shows the antistreptolysin content of the sera of 41 pa-

TABLE I.

Antistreptolysin content in forty-one cases of recurrent tropical lymphangitis.

Case	Antistreptolysin units	Case	Antistreptolysin units
F. M.	83	J. N. C.	406
C. F.	1041	S. R.	133
J. C.	252	F. C.	117
M. F.	219	C. M.	208
A. L. P.	83	Z. C.	606
M. E. R.	170	A. I.	76
F. S.	333	D. C.	138
L. C.	166	P. A.	151
M. L.	83	C. V.	238
P. G. S.	269	F. O.	151
F. A.	444	M. F.	208
E. O.	104	P. M.	151
J. M.	231	M. I.	138
A. A.	231	M. T.	277
P. L.	277	N. R.	138
P. J.	256	E. H.	538
E. A.	83	D. M.	79
D. A.	333	G. R.	98
G. O.	98	N. C.	1111
D. R.	208	P. M.	437
C. J.	92		

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[3] Hodge, B. E., and Swift, H. F., *J. Exp. Med.*, 1933, **58**, 277.

tients suffering from attacks of recurrent tropical lymphangitis. In these cases, sera were obtained during the attack, few days after the attack and in some instances several weeks after the acute attack, but no classification in this respect will be attempted at this time.

TABLE II.
Antistreptolysin content of the sera of normal subjects.

Case	Antistreptolysin units	Case	Antistreptolysin units
P. M. O.	138	S. F.	62
C. L.	46	L. H.	64
N. R.	151	A. R.	52
J. F.	22	J. M.	28
A. P.	25	R. M.	21
J. A. P.	52	C. F.	62
E. R.	66	A. G.	62
M. O.	64	L. G.	111
A. L.	18	E. S.	46
A. C.	46	E. C.	151

Table II shows the results obtained with the sera from 20 apparently normal individuals.

In 2 cases studied during and after the acute attack we find the following: In a case having attacks of acute lymphangitis at monthly intervals for the last 2 years, the antistreptolysin titer 5 days after the attack was 606 units. Thirty-five days after the attack 538 units and 60 days after the attack 333 units. A chronic case of 15 years' duration had attacks spaced at long intervals and has had no attacks for the last 20 months. During an acute attack the antistreptolysin titer was 100 units during the attack, 71 units 11 days after the attack and 47 units 35 days after.

Summary. The antistreptolysin values of the sera of 41 cases suffering from recurrent tropical lymphangitis and 20 normal cases are here reported. Apparently the antistreptolysin titer is increased in cases of lymphangitis if compared with normal subjects. The serum of cases suffering from acute lymphangitis varies in antistreptolysin titer according to whether the serum is studied before, during or after the attack.

7488 C

I. Effect of Dyes on Colonies of Certain Pathogenic Fungi.

JOHN W. WILLIAMS.

From the Department of Biology and Public Health and the Homberg Memorial Infirmary, Massachusetts Institute of Technology.

In the present work observations were made of the growth of certain pathogenic fungi on a medium containing alcoholic nigrosine and one containing litmus.*

The medium† consisted of 4% peptone, 1% dextrose, 1½% agar in distilled water and was adjusted to pH 5.5 and saturated with dye by heating, filtered and autoclaved. Sabouraud's proof medium and a control medium without dye also were planted. The dyes were incorporated in the 4% peptone, 1% dextrose medium instead of Sabouraud's proof medium since we felt that with this medium moisture was retained better, there was better differentiation, growth was as rapid and slightly more profuse and, as a rule, less aerial. In many instances the growth showed a frosted surface effect resembling the growth of yeasts which type of growth was absent in the case of Sabouraud's proof medium. Difco products were used.

The following pathogenic fungi and 2 saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis* were observed: *Achorion schoenleinii, Acladium castellani, Candida candida, Endodermophyton tropicale, Endomyces capsulatus, Endomyces dermatitidus, Epidermophyton inguinale, Glenospora gammeli, Geotrichum bachmann, Indiella americana, Monosporum apiospermum, Microsporon audouini, Monilia albicans, Oöspora humi, Trichophyton crateriforme, Trichophyton granulosum, Trichophyton japonicum, Trichophyton interdigitale, Willia anomala.* Observations were limited to 30 days. The tubes were kept at room temperature and growth occurred in all within 3 days.

The table gives the time within which color was noted. t indicates tint, m mycelial, s slight, f frosted. In the litmus medium several growths appeared whiter and these are so designated. The microscopic color change where recognizable is designated by t.

The results are apparent from the table. The growth of *Indiella*

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* Black Nigrosine (alcohol soluble), Central Scientific Co., Boston, Mass. Litmus Cubes, Merck & Co., New York.

† Details of Medium in future publications entitled: "Studies of the Effect of Variation of the Ratio of Peptones to Dextrose on the Colonies of Certain Pathogenic Fungi."

TABLE.

Organism	Nigrosine Medium color		Litmus Medium color	
	Macroscopic	Microscopic	Macroscopic	Microscopic
Achorion schoenleinii	7	t	7	t
Acladium castellani	11	t	11	t
Candida candida	14	t	11	t
Endodermophyton tropicale	11 t	t	11 t	t
Endomyces capsulatus	11 f	t	whiter	
Endomyces dermatitidus	20 f		20 f	
Epidermophyton inguinale	20 t m		20 t m	
Geotrichum bachmann	7	t	7	t
Glenospora gammeli	20 f	t	whiter	
Indiella americana				
Lichtheimia sp.				
Monosporum apiospermum	24 f	t	24 t f	
Microsporon audouini	24 t		24 t	
Monilia albicans	11	t	11	t
Oöspora humi	11	t	20	t
Scopulariopsis brevicaulis	20 t	t	20 t	
Trichophyton crateriforme	14 t m	t	20 t m	t
Trichophyton granulosum	14 t m	t	20 t m	t
Trichophyton interdigitale	14 t m	t	20 t m	
Trichophyton japonicum			whiter	
Willia anomala	7	t	11	t

americana on Sabouraud's was a light yellow, on all other media white, with the exception of a small frosted rim which took the color of nigrosine and litmus in the respective instances. Sabouraud's proof medium did not show the frosted growth appearance, while in the control medium it was especially apparent with *Endomyces capsulatus*, *Endomyces dermatitidus*, *Glenospora gammeli* and *Monosporum apiospermum*.

7489 C

II. Effect of Dyes on Colonies of Certain Pathogenic Fungi.

JOHN W. WILLIAMS.

From the Homberg Memorial Infirmary and the Department of Biology and Public Health, Massachusetts Institute of Technology.

In a previous paper observations were made on the growth of certain pathogenic fungi on a medium containing alcoholic nigrosine and one containing litmus.[1] In the present paper a like medium (peptone 4%, dextrose 1%, agar 1½%) containing 2% Eosin Y and one containing 2% Eosin B* were used.

The following pathogenic fungi and 2 saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis* were observed: *Achorion schoen-*

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[1] Williams, John W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1173.

leinii, *Acladium castellani*, *Candida candida*, *Endodermophyton tropicale*, *Endomyces capsulatus*, *Endomyces dermatitidus*, *Epidermophyton inguinale*, *Glenospora gammeli*, *Geotrichum bachmann*, *Indiella americana*, *Monosporum apiospermum*, *Microsporon audouini*, *Monilia albicans*, *Oöspora humi*, *Trichophyton crateriforme*, *Trichophyton granulosum*, *Trichophyton interdigitale*, *Trichophyton japonicum*, *Willia anomala*. Observations were made over 30 days. The tubes were kept at room temperature.

Growth occurred within 4 days in all tubes of the control media and in the dye tubes with the exception of *Achorion schoenleinii*, *Endodermophyton tropicale*—Eosin B, *Glenospora gammeli*, *Microsporon audouini*—Eosin Y where it occurred in 6 days, *Endodermophyton tropicale*—Eosin Y, *Microsporon audouini*—Eosin B, where it occurred within 11 days and *Trichophyton crateriforme*, *Trichophyton japonicum*—Eosin B, where it occurred within 8 days. Growth was as a rule somewhat less marked on the media containing dye but only markedly so in the instance of *Trichophyton japonicum*.

As in the previous article[1] the frosted appearance was noted in the colonies of the control medium and Eosin Y medium with *Endomyces capsulatus*, *Endomyces dermatitidus*, *Glenospora gammeli*, *Indiella americana*, *Monosporum apiospermum* and in Eosin B medium with *Endomyces capsulatus*, *Endomyces dermatitidus* and *Indiella americana*. A narrow frosted rim was noted with *Trichophyton japonicum* on Eosin Y.

Color was marked in the organismal colonies on practically all the dye media. *Candida candida* appeared more granular in the presence of dye, *Epidermophyton inguinale* less wrinkled, *Monilia albicans* more granular on Eosin B, *Oöspora humi*, more mycelial. Macroscopic color was observed in all growths within 7 days with the exception of *Endodermophyton tropicale*—Eosin Y, *Epidermophyton inguinale*, *Trichophyton interdigitale*, where color was noted within 11 days and *Indiella americana*, *Lichtheimia sp.*, *Microsporon audouini*—Eosin B, where color was observed within 20 days. The color made its first appearance in the center of the colony of *Trichophyton interdigitale* proceeding from there to the outer borders of the colony.

On microscopic examination each element of the different colonies seemed capable of taking up color. The mycelia, however, in general showed less tendency to take up color. The depth of color and the number of elements colored varied with the growth. A more uniform tinting was present in some of the more mycelial growths. In most cases granules, vacuoles and refractile rims were very apparent. There were no indications that the more deeply colored elements lacked viability. Both eosins gave good results.

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* Eosin Y (water and alcohol soluble), Schultz No. 587, Batch NE-7, National Aniline and Chemical Co., Inc., New York, N. Y.

Eosin B (water and alcohol soluble), Schultz No. 590, Batch NEb-4, National Aniline and Chemical Co., Inc., New York, N. Y.

It is felt that the dye media used are of value in differentiation and that the added expense of this type of vital staining is compensated for by the greater distinctness of appearances.

7490 C

Growth of Certain Pathogenic Fungi on Asparagin Medium.

JOHN W. WILLIAMS.

From the Department of Biology and Public Health and the Homberg Memorial Infirmary, Massachusetts Institute of Technology.

Since asparagin is used frequently as a substitute for peptone, it was substituted in the following observations and the differential characteristics of the organisms planted were observed.

The media used differed in that a portion contained 1% dextrose while another portion did not. The constituents were: Asparagin 10 gm., dextrose 10 gm., $MgSO_4.7H_2O$ 0.4 gm., NaCl 2.0 gm., KH_2PO_4 6.0 gm., K_2HPO_4 1.0 gm., agar 1.5 gm., distilled water 1000 cc.

The salts as used resulted in a pH of 5.5. A control medium of 4% peptone, 1% dextrose and 1½% agar was planted.

The following pathogenic fungi and 2 saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis* were observed: *Achorion schoenleini, Acladium castellani, Candida candida, Endodermophyton tropicale, Endomyces capsulatus, Endomyces dermatitidus, Epidermophyton inguinale, Glenospora gammeli, Geotrichum bachmann, Indiella americana, Monosporum apiospermum, Microsporon audouini, Monilia albicans, Oöspora humi, Trichophyton crateriforme, Trichophyton granulosum, Trichophyton interdigitale, Trichophyton japonicum, Willia anomala.* Observations were made over 30 days. The tubes were kept at room temperature.

The table gives the number of days within which growth occurred. One plus indicates a scant growth, 2, 3, 4 plus successively larger growth, and 5 plus the entire slant covered; v plus indicates very scant, a growth which we have been able to duplicate on plain agar. In no instance were the control growths less than 4 plus nor was their appearance delayed more than 4 days. There was slightly greater growth on the asparagin dextrose than on the control medium with *Endomyces capsulatus, Glenospora gammeli, Monosporum apiospermum.* A frosted appearance occurred on the control medium as stated in previous papers[1, 2] with *Endomyces capsulatus, Endomyces dermatitidus, Glenospora gammeli, Indiella americana, Microsporon apiospermum* and on the asparagin dextrose medium with *Endomyces dermatitidus.*

TABLE I.

Organism	Asparagin	Asparagin dextrose
Achorion schoenleinii	8 +	8 ++
Acladium castellani	4 +	4 ++++
Candida candida	4 ++	4 +++
Endodermophyton tropicale	15 +	15 +
Endomyces capsulatus	15 +	8 +++++
Endomyces dermatitidus	15 v +	8 +++++
Epidermophyton inguinale	11 +	8 ++++
Glenospora gammeli	11 +++	6 +++++
Geotrichum bachmann	6 +++	4 +++++
Indiella americana	11 v +	6 +
Lichtheimia sp.	11 v +	11 v +
Monosporum apiospermum	8 +++	4 +++++
Microsporon audouini	8 ++	8 +++
Monilia albicans	4 ++	4 ++
Oöspora humi	4 +	4 ++++
Scopulariopsis brevicaulis	4 +++	4 +++++
Trichophyton crateriforme	8 +	4 +++
Trichophyton granulosum	4 +++	4 +++++
Trichophyton interdigitale	15 +++	4 +++++
Trichophyton japonicum	11 +	4 ++
Willia anomala	4 ++	4 ++++

The type of growth on the asparagin medium varied from that of the control medium in many instances; with *Achorion schoenleinii,* that on asparagin dextrose was finer and more dendritic; with *Acladium castellani,* that on both asparagin media more discreet; with *Candida candida,* subsurface mycelia were marked on both asparagin media; with *Geotrichum bachmann,* the growth on asparagin appeared glassy; with *Indiella americana,* there were subsurface mycelia; with *Trichophyton crateriforme* on asparagin the growths were discreet. In general, where there was any considerable growth the mycelia were more compact and whiter. The difference in amount of growth is apparent from the table.

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[1] Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1173.

[2] Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1174.

7491 P

Structural Changes in Adult Pituitary after Injecting Extracts of Castrate or Menopause Urine.*

AURA EDWARD SEVERINGHAUS.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Injection of oestrin[1] or of P.U. extract[2] into adult rats has been shown to decrease the gonad stimulating potency of their pituitaries. Marked pituitary changes, consisting primarily of degranulation and retrogressive changes in the basophiles, occur in the pituitaries of P.U. injected rats.[3] These structural findings are of such character as might be expected in cases of decreased potency.

The present paper is concerned with anterior pituitary changes in the adult female rat following prolonged injection of follicle stimulating hormone (hereafter spoken of as C.U.) from the urine of castrate or menopause women. Smith and Engle in recent and yet unpublished experiments have shown a marked decrease in the gonad stimulating potency of the pituitary of the adult female rat when injected with C.U. Ovarian weights of immature littermate female mice which received pituitary implants from normal or injected animals were taken as the measure of potency. The potency of pituitaries from injected rats was strikingly reduced, in many instances averaging about one-half that of the normal. Equivalents of 40 cc of urine were injected daily for from 27 to 42 days.

Cellular changes in the anterior pituitary following C.U. injections are striking. Of the 3 cell types, the basophiles are most affected. A marked degranulation occurs in many cells. With the cytological technique which we employ, the blue granules are depleted, but are frequently replaced in part by irregular cytoplasmic masses which stain yellow. This phenomenon is the same, though more accentuated, as that previously described[3] in pituitaries from P.U. injected animals. The degranulated cells, at times, have a cytoplasm free of visible structure, except for the Golgi sphere which retains its blue ground color dotted by minute acidophilic granules.

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council, administered by Dr. P. E. Smith.

[1] Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 702; *Endocrinology*, 1932, **16**, 655.

[2] Leonard, S. L., *Anat. Rec.*, 1933, **57**, 45.

[3] Severinghaus, A. E., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 593; *Anat. Rec.*, 1934, **60**.

In other cells, mitochondria may be present in varying abundance although the blue granules have been depleted. These pituitaries show also the numerous small basophilic cells with prominent enlarged Golgi. They are believed to be newly formed basophiles which become active before reaching normal full size.

An additional feature of interest is the unfailing presence in our C.U. injected animals of branching areas of deeply-staining degenerate cell masses. These areas are greatest in our longest treated animals. Examination shows that these areas are made up to a large extent of angular, shrunken, basophilic cells with deep blue, pyknotic nuclei and a dense, granule-free, blue cytoplasm, against which the Golgi ring is prominently contrasted. Small branching areas of degenerating cells have been seen upon occasion in supposedly normal pituitaries, especially of older rats, but not to the extent that this condition occurs in the injected animals. The basophilic degranulation, as well as the large inclusions of degenerated basophile cells, correlates well with the greatly decreased potency of the pituitaries of C.U. injected rats and with previous potency and cytological findings on pituitaries of P.U. injected rats.

The large number of degranulated basophiles and the many small cells of definite basophilic character would, with many techniques, give the appearance of a proportional increase in chromophobes, a finding in our opinion not real but apparent. The acidophiles are quite normal in size and character, but the impression of a reduction in their number, sometimes much more marked than at others, would doubtless be supported by a statistical study.

7492 P

Phosphatase Studies. VIII.
Increase of Serum Phosphatase After Bile Duct Ligation in Dog.

AARON BODANSKY AND HENRY L. JAFFE.

From the Laboratory Division, Hospital for Joint Diseases, New York City.

Roberts has shown increased plasma phosphatase in obstructive jaundice.[1, 2] We have demonstrated an increase of serum phosphatase in a series of cases of catarrhal jaundice, and a return to normal values after their clinical improvement.[3] We interpreted

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[1] Roberts, W. M., *Brit. J. Exp. Path.*, 1930, **11**, 90.

[2] Roberts, W. M., *Brit. Med. J.*, 1933, **1**, 734.

these data as supporting the assumption of a hepatogenic source of serum phosphatase. It seemed desirable to study the subject experimentally—by bile duct ligation in one group of experiments.

The operation was performed under amytal anesthesia upon a young adult female dog weighing 11.6 kilos. The dog made a good recovery. Observations covered a period of over 7 weeks. About 4 weeks after the operation, the dog having had repeated attacks of vomiting, it was decided to discontinue food for a few days and then to resume feeding with certain precautions. Advantage was taken of this period of fasting in order to compare the results with earlier observations on the effects of fasting on normal dogs.[4]

TABLE I.
Serum Inorganic Phosphorus, Phosphatase, Icteric Index and Bilirubin after Bile Duct Ligation.

Date 1934	P mg.	E units	Icteric Index	Bilirubin mg.	Notes
1/21	3.1	4			Operated
1/30	3.2	133	39	3.1	
2/18	4.3	200	49	5.6	Fast begun
2/19	4.6	188	45		Fast continued
2/20	4.6	187	55	4.3	” ”
2/21	4.6	155	50	4.0	” ”
2/23	4.0	139	57	4.6	Fast discontinued
2/28	3.8	193	48	3.6	
3/8	3.6	330	40	5.9	Bloody stools
3/10	11.0	320	40	7.9	Moribund

P—inorganic phosphorus (mg. per 100 cc.); E—phosphatase (units per 100 cc.).

At the time of the last analysis the inorganic phosphorus determination indicated impairment of kidney function. This indication was supported by the findings of 51.7 mg. of urea N per 100 cc. of blood and of 2.1 mg. of creatinine.

At the same time the uric acid content of the blood was 4.8 mg. per 100 cc.; in dogs with normal liver function the uric acid content of blood is too low to be determined. The dog was killed by administration of ether. Immediate necropsy revealed multiple areas of liver necrosis.

The rise of serum phosphatase was not parallel to the increase of the icteric index or of serum bilirubin. This, as well as other considerations, indicate that the increases of serum phosphatase, of the icteric index and of bilirubin represent disturbances of different functions of the liver. It is also to be noted in this connection that

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[3] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 107.

[4] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 199.

during the period of fasting serum phosphatase decreased from 200 to 139 units; the changes of icteric index and of bilirubin were not parallel during the same period or subsequently.

The maximum phosphatase content was approximately 80 times the initial value. This is the highest value that we have obtained in any condition in any animal. It may well be near the possible maximum in the dog. The high value of serum phosphatase was all the more significant in view of the marked malnutrition toward the end of the experiment, associated with gastro-intestinal disturbances and hemorrhages (necropsy showed gastric and intestinal ulcers).

It would have been desirable to amplify this study with data of tissue phosphatase changes. Studies in this direction are in progress.

7493 P

Pattern Analysis in Plumage.* I. Curve of Barb Growth.

MARY JUHN AND RICHARD M. FRAPS.

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

Lillie and Juhn[1] reached the conclusion that "general physiological conditions produce morphological diversity in genetically identical feather germs, and . . . . the main cause of the diversity of action is definitely oriented, accurately regulated differences of growth rate within the germ." (p.177.) It was further pointed out that, since pattern is primarily differentiation in respect of barb level, the curve of barb growth rate becomes highly important in the interpretation of pattern. A curve given in the earlier study was believed to describe the growth of a barb from its origin at the ventral collar limit to its emergence on the rhachis. From this curve of barb growth it was concluded that the rate of barb growth decreased continuously from a maximum value at the point of origin to a minimum value at the dorsal limit of the collar. Differentials in growth rate are greatest through the ventral-most region of the collar and decrease progressively to the dorsal limit of the collar. Growth rate through the ventral-most quarter of the collar is, in terms of the curve of barb growth given, several times the rate of growth through the 2 dorsal fourths of the collar.

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* The expense of this investigation was supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

[1] Lillie, Frank R., and Juhn, Mary, *Physiol. Zool.*, 1932, **5**, 124.

Further study of the subject has confirmed the idea of a growth gradient in the barbs, but at the same time has led to the conclusion that the gradient was originally represented as too steep from ventral to dorsal regions. Several lines of evidence bearing on this conclusion are as follows:

1. Completely limited pattern reactions. Lillie and Juhn have shown that it is possible to induce the female pattern completely across one vane-half of wing covert feathers without visible reaction in the opposite vane-half. This complete asymmetry is interpreted in terms of differential growth rate levels of barbs on the 2 sides of the germ. It follows that there can have been no regions of equal growth rates in the 2 collar halves during the period of pattern determination. The maximum rate of barb growth in the vane-half reacting to female hormone must accordingly have been less than the minimum growth rate of the vane-half in which the reaction does not appear.

The limiting necessary difference of growth rates in the 2 collar halves is given approximately by the differences in growth rate at dorsal and ventral limits of the generalized curve of barb growth. Assuming moderate values for the ventral-dorsal ratio of barb growth rates, the difference in rates of growth of barbs in the 2 collar halves would be of the order of several hundred percent. Known relations of collar length, barb length and asymmetry of growth observed in germ preparations combine to eliminate the possibility that growth rate differences of any such order exist in fact in the wing covert feather.

2. Making use of the methods described in the following note, we have attempted to transpose pattern relations in the definitive feather to events in the collar which must have corresponded to the origin of these patterns.

The regions of reaction observed immediately adjacent to the collar correspond with the observed pattern in the definitive feather. If, however, we attempt to follow the course of a pattern above the zone of differentiation, and from these relations attempt a transposition to the definitive feather or vice versa, discrepancies are apparent. The transposition of the definitive feather pattern indicates that the differences in growth rates in the germ in respect of barb level are relatively small. A quantitative formulation of barb development from the time of origin of the barb to its final position in the feather should make possible a direct transposition of pattern relations from the definitive pattern to the germ and equally, the converse operation. We are at present engaged in such an analysis.

3. Fault bars. The ideal line of pattern contour would of course be given if an instantaneous reaction might be induced at the same collar level with respect to the barbs. An examination of fault bars suggests that they may approach this limiting condition rather more closely than do experimentally induced reactions. We have attempted to transpose the definitive fault bar line to the collar in order to derive the antecedent barb relations. The *apparent* differences in barb growth rates thus arrived at are smaller than are the differences called for by the original curve of barb growth. At this time we record these observations without considering the value of fault bars in plumage analysis to have been demonstrated beyond question.

7494 P

Pattern Analysis in Plumage. II. Methods of Definitive Feather Analysis.

MARY JUHN AND RICHARD M. FRAPS.

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

The correspondence of events in the germ and definitive feather pattern characteristics is implicit in the relations which Lillie and Juhn have shown to exist in the collar during the reaction of barbs at all levels to high concentrations of thyroxin and female hormone. From this point of view measurable elements of pattern are of direct developmental and physiological significance. Definitive pattern relations are also of direct importance in other respects, as in the quantitative formulation of symmetry relations. We report here methods which have been developed to give the desired data.

The characteristics of the definitive feather which can be measured are distances between barbs on rhachis, lengths of barbs, and lengths of pattern elements within the limits of definition as barb or rhachis segment. Barbule lengths and distances between barbules may also be measured in certain instances.

(a) Mounting the feather. In order to obtain the desired measurements on barb length and barb frequency with precision and reasonable rapidity it is necessary that the feather be permanently mounted with reference to simplest possible axes. *i. e.*, with barbs at right angles to rhachis.

The rhachis of the feather is first set in paraffine, applied hot, on the glazed surface of heavy bristol board. The barbs are then brushed out until they lie approximately at right angles to the rhachis. The bristol board is now clamped down on a base movable in the line of and exactly parallel to the rhachis. Above this sliding frame and at right angles to its line of motion is a guide bar. With a brush kept hot in molten paraffine the barbs are now pulled into position exactly at right angles to the rhachis; in the final operation the brush is held against the transverse guide bar and drawn slowly from rhachis to margin. Surplus paraffine is removed at the same time. The paraffine, when cooled, holds the barbs in place. In measuring distances between barbs the junction of barbs and rhachis must be free of paraffine. This is accomplished by running a small heated metal bar along the rhachis until excess paraffine has flowed completely out of all junctions. The mounted feather may be given a coat of shellac or euparal for permanent protection; if the specimen is to be used for measurement of barb spacing, junctions of barbs and rhachis should be left free.

(b) Barb frequency. Barb frequency is defined as the number of barbs per unit of rhachis length; it is therefore the reciprocal of the distance between 2 barbs, or the reciprocal of average distance between barbs for any number of barbs. The direct datum is of course the distance at the rhachis between 2 or more barbs.

For determination of distance between barbs the mounted feather is clamped down on a stage with the rhachis parallel to the line of movement of the gauge. The gauge carries a needle pointer which may be set at any point on the length of the rhachis. Minor adjustments for exact coincidence of pointer and barb junction are made by changes in the position of the stage carrying the feather. A binocular microscope, travelling in the direction of the gauge axis, is used in making all settings of the pointer.

Two gauges are mounted in combination for various requirements: a micrometer reading directly to 0.01 mm. is used for measuring distances between 2 barbs, etc. By estimation, differences in length of 0.002 mm. are easily determined. The micrometer carries the pointer. The second gauge is a vernier reading to 0.02 mm. It is used in measuring distances between a sufficient number of barbs, barb lengths, etc. The micrometer is clamped directly to the vernier. Either gauge may be used independently by locking the other, or both gauges may be used, as for example in measuring the distance between barbs at given intervals along the rhachis.

(c) Length of barbs and length of pattern segments. The feather mount is fixed upon the stage with rhachis at right angles to the gauge axes. The barbs then, are parallel with these axes. The gauge pointer can be set for any point along the axis of a barb as in the measurement of barb distances it was set at required points along the rhachis. Measurements are made progressively from apex of barb through all intervening pattern loci to junction of barb and rhachis. The stage carrying the feather is free to move in the line of the rhachis; any barb on the rhachis can be set under the pointer of the measuring gauge.

Measurement of barb frequency, barb length, and length of pattern segment with these methods becomes a relatively simple operation. A very high degree of accuracy is easily attained. Barbule frequencies and barbule lengths have been determined in some instances, although not to the degree of accuracy possible in the determination of barb and rhachis characteristics.

Determination of barb frequencies in various feathers points to a characteristic form of curve in the several feather tracts. Curves for breast differ greatly from curves for saddle and back. Some evidence is at hand indicating orderly relations of barb frequency to asymmetries of feather and tract. These general relations will be reported in detail elsewhere.

7495 P

Pattern Analysis in Plumage. III. Action of Thyroxin in High Concentrations.

MARY JUHN AND RICHARD M. FRAPS.

From the Whitman Laboratory of Experimental Zoology, University of Chicago

The sequence of response of the components of the definitive feather to increasing thyroxin concentrations—blanching of barbs, barbule formation, and pigment deposition—and the manner of estimating barb growth in the germ by observation of the order of appearance of pigment following injection of thyroxin have been established by Lillie and Juhn.[1]

An examination of definitive feathers subjected to heavy thyroxin dosages (10 mg., single injection) during development shows that both barb frequency and barb lengths are markedly altered. Barb frequency increases to a level around twice the barb frequency of a normal feather; a sudden drop then occurs to a level below that

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[1] Lillie, Frank R., and Juhn, Mary, *Physiol. Zool.*, 1932, **5**, 124.

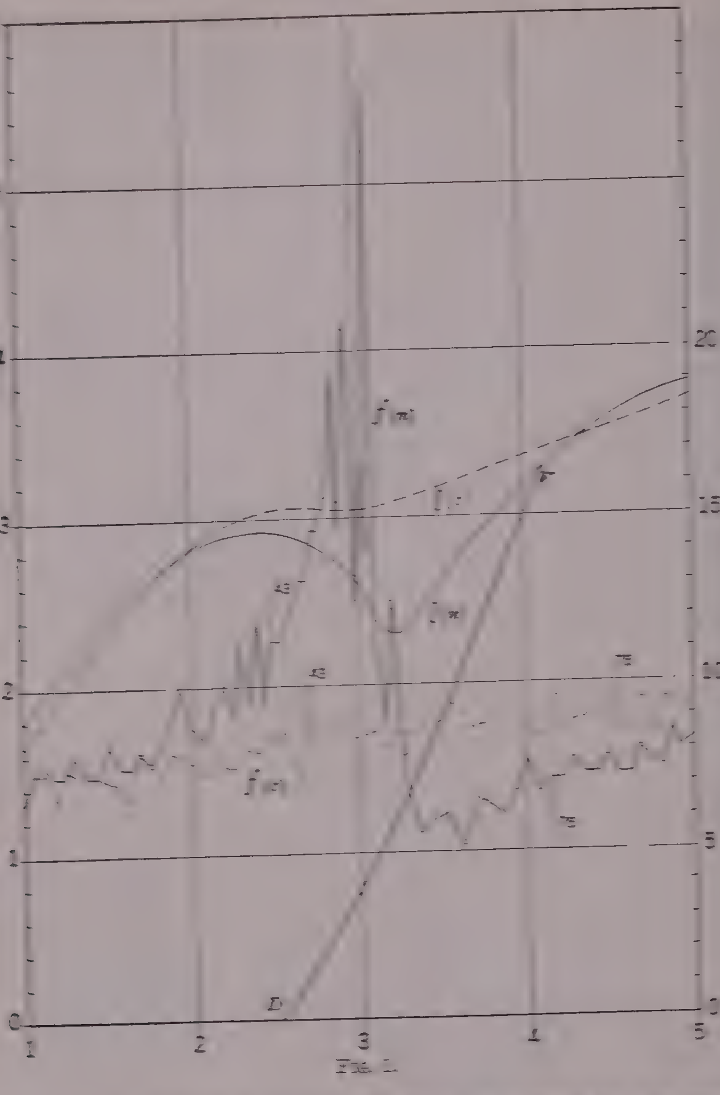

FIG. 1.

characteristic of barb frequency for the same region of the control feather. Barb length decreases following injection of thyroxin, then gradually returns to normal or possibly greater than normal length.

These relations are shown in the accompanying graph. The curves are for single longitudinal vane-halves of modified and control neck feathers. Distances from origin of rhachis are given in centimeters on the horizontal axis. Figures on the left ordinate refer to barb frequency, *i. e.*, the number of barbs per millimeter rhachis length. Figures on the right ordinate apply to barb lengths.

Barb frequency of the control and modified feathers are given by the curves $f_{(c)}$ and $f_{(m)}$ respectively. Curves $l_{(c)}$ and $l_{(m)}$ are contour lines of the control and modified vane margins respectively. The length of barb at any point on the rhachis is the distance from the contour line to the base of the figure.

The diagonal line, D-V, is the line of pigment deposition; the segment of barb lying between D-V and the contour line $l_{(m)}$, is the length of barb above the collar at the time of pigment deposition. Lengths between D-V and the rhachis (abscissa) are barb segments laid down following pigment determination in the collar.

The numbers "43" and "75" refer to identical barbs on the modified and control rhachises. The region of pigment deposition extends from barb 43 to barb 75. Due to the modified barb distribution along the rhachis of the feather treated with thyroxin, these and all intervening barbs—which in at least this number were in the collar at the time of thyroxin injection—must be compared with corresponding barb number in the control feather.

Both barb frequency and barb length are modified by thyroxin over a considerable range anterior to the first barb showing pigment deposition. This effect is possibly due either to failure of thyroxin to effect melanin deposition in all barbs in the zone of determination, or to interference with the normal expansion (ballooning) of barbs and rhachis after they have passed beyond the zone of determination.

The significance of these results in relation to events in the germ will be taken up in a forthcoming paper; at that time we shall consider also the action of thyroxin over a wide range of concentrations.

7496 P

Pattern Analysis in Plumage. IV. Order of Asymmetry in the Breast Tracts.

MARY JUHN AND RICHARD M. FRAPS.

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

In experiments with female hormone in this laboratory it was found that when the mark due to the hormone was restricted to one vane-half in the definitive breast feather, other feathers were occasionally found in the same tract bearing a similar mark on the opposite vane-half. There are nine feathers in a transverse row across each breast tract; counting laterally from ventral mid-line of the bird, we shall designate the feathers of such a row by the numbers one to 9. Ordering the various feathers in which the asymmetrical mark was found according to this notation, it could be shown that feathers one to 5 bore the female hormone mark predominantly on one vane-half; feathers 7, 8 and 9 carried the mark predominantly on the opposite vane-half. Feather number 6 in this composite row showed little if any asymmetry in distribution of the female hormone mark, *i. e.,* both vane-halves were marked to about the same degree. Finally, the degree of asymmetry increased from feather to feather in the order of distance from number 6: the greatest asymmetry of marking thus occurred in those feathers nearest the ventral and lateral margins of the breast tract, but of course on opposite vane-halves.

These observations pointed to a true reversal of asymmetry within each breast tract with respect to a secondary antero-posterior axis of symmetry. The position of the secondary axis was apparently given by the sixth feather of each transverse row. The precision of response occasionally observed indicated, moreover, that the relations of asymmetry of feathers ventrally and laterally from the assumed secondary axis of symmetry were of an exact quantitative order.

An examination of the order of appearance of feather papillae (Anne Holmes, in preparation) has shown that the first line of papillae to arise in the embryo corresponds with the line of follicles of most symmetrical reaction pattern, which in the breast is the line of follicles numbering 6 in the transverse row.

In this note we give the results of measurements of barb lengths of definitive breast feathers which demonstrate that, in respect of barb length the order of asymmetry in the breast tracts is essentially in accord with that deduced from our observations on response to female hormone.

Barb lengths are measured at 0.5 cm. intervals on both sides of the rhachis from one cm. below the tip of the feather to within one cm. of the end of the fluff. The relative asymmetry of each feather of a transverse row is calculated by 3 procedures. 1. The differences in lengths of barbs on opposite sides of the rhachis are averaged for all measurements made. 2. The maximum difference in length of barbs found down the axis of the feather is taken as the index of asymmetry. 3. The average difference in barb lengths over the cm.

showing the greatest differences in barb lengths. In practically all cases the 3 "indices" are in agreement, although of course differing in absolute magnitude.

The relations of asymmetry in the breast tracts, as established by these results, are given in the chart. A′—A′ represents the mid-ventral line of the bird. Passing laterally to right and left from the ventral mid-line, figures 1′, 2′, . . . 9′, give the positions of the feathers in right and left transverse rows.

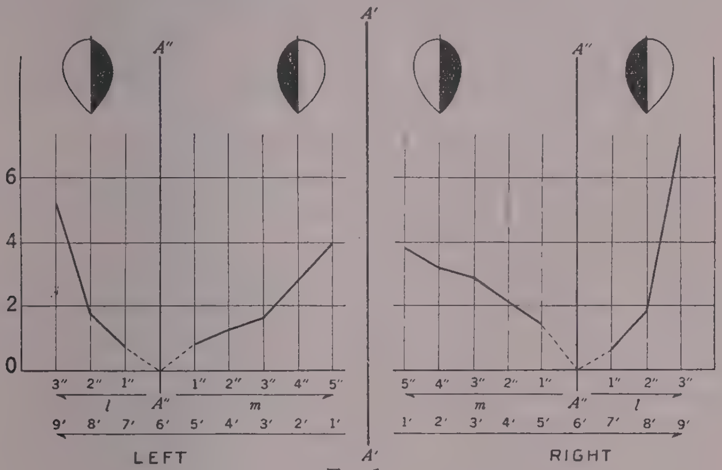

Fig. 1.

The index of asymmetry chosen is the average difference in barb lengths over the cm. of greatest difference in barb lengths. The feather showing the smallest absolute difference in length of barbs determines the secondary axes, A″—A″, right and left. This is the feather number 6 of the primary order. From the secondary axes, A″—A″, the feathers of the transverse rows are re-ordered as shown in the chart; m and l refer to feathers medially and laterally respectively for each breast tract.

The "indices" of the remaining feathers of each tract are now ordered with respect to the secondary axes. Ordinates in the chart refer only to the differences in barb lengths taken with respect to these axes, *i. e.*, with the feather viewed from the ventral surface, base up, the length of barbs in the vane-half adjacent to the secondary axes is subtracted from the length of barbs in the vane-half away from the secondary axes. These vane-halves may be spoken of as adaxial and abaxial respectively.

Abaxial barb lengths, then, are greater than adaxial barb lengths over the cm. of greatest differences by the amounts shown in the chart.

We have some evidence that the sixth feather of the primary order is not necessarily the feather of least asymmetry in all instances. But once having established the secondary axis for a right or left breast tract, we have found no exceptions to the order of adaxial-abaxial relationship. Also, the absolute value of differences in barb lengths is subject to considerable variation; the general nature of the relations shown in the chart, however, remains the same, with extreme asymmetry at the lateral boundaries of the tract.

Missouri Section.

Washington University Medical School, May 9, 1934

7497

Skin Lesions Produced by Intradermal Inoculation with Hemolytic Streptococci.

PAUL O. HAGEMAN. (Introduced by Jean V. Cooke.)

From the Department of Pediatrics, Washington University School of Medicine, St. Louis.

To determine whether any strain specificity could be shown in freshly isolated hemolytic streptococci from erysipelas as compared with those from other sources, intradermal injections into rabbits were made with 10 strains from erysipelas, 10 from scarlet fever, 3 from acute abscesses, 2 from meningitis, and 5 stock strains, including 2 originally isolated from acute mastoiditis, and the scarlatinal strains NY 5 and Dick II, and erysipelas strain A I furnished by the New York City Department of Health Laboratories. The dosage was 0.02 of an emulsified 24-hour blood agar slant injected into the depilated skin of the back, 2 rabbits being tested with each strain. In no instance did a spreading erysipelas develop, the lesion produced being a localized reddened indurated swelling involving from one to 10 sq. cm., reaching its height on the second or third day, and subsiding slowly. Occasionally an abscess formed. The results are summarized in Table I.

Although the appearance of the lesion was identical with all organisms used, the strains from erysipelas produced slightly larger areas of skin involvement, the average size of the lesion produced by erysipelas strains being 3.55, that of scarlet fever strains 1.66, and that of the organisms from septic lesions 1.35 expressed as square centimeters. The stock strains used showed lesions similar in size and character to those produced by freshly isolated organisms from septic infections.

Three strains from scarlet fever and 3 from erysipelas used intradermally were each passed through 3 rabbits. After such passage the skin lesions produced by the erysipelas strains were of approx-

TABLE I.

Scarlatinal		Erysipelas		Miscellaneous		
Size of skin lesion in mm.	Abscess Formation	Size of skin lesion in mm.	Abscess Formation	Source	Size of skin lesion in mm.	Abscess Formation
10 x 10	—	30 x 15	+	Meningitis	10 x 10	—
10 x 10	—	20 x 15	—		10 x 10	—
15 x 15	—	25 x 20	+	Adenitis	15 x 10	—
10 x 10	—	20 x 20	+		10 x 10	—
10 x 10	+	25 x 20	+	”	15 x 10	—
15 x 15	—	35 x 30	+		10 x 10	—
25 x 25	+	15 x 15	—	Meningitis	10 x 10	—
15 x 15	—	15 x 15	—		10 x 10	—
10 x 10	—	20 x 20	—	Abscess	20 x 15	+
10 x 15	—	30 x 25	+		15 x 10	—
15 x 10	+	15 x 10	—	Stock Mastoid 80A	10 x 10	—
10 x 10	—	15 x 10	—		10 x 10	—
15 x 10	+	30 x 25	—	Stock Dick II	15 x 10	—
10 x 10	—	20 x 20	—		15 x 10	—
10 x 10	—	15 x 15	—	Stock A I Birkhaug	15 x 15	—
10 x 10	—	10 x 10	—		15 x 15	—
15 x 15	+	10 x 10	—	Erysipelas Stock N. Y. 5	10 x 10	—
15 x 10	—	10 x 10	—		10 x 10	—
15 x 10	—	15 x 15	+	Stock Mastoid 80B	10 x 10	—
15 x 10	—	10 x 10	—		10 x 10	—

imately the same size as before and averaged 4.5 sq. cm. Each of the scarlet fever strains was found to involve a larger skin area than previously, the average size being 4.8 sq. cm., or an increase to the size of the lesion resulting from the erysipelas organisms.

While the experiments indicate that streptococci freshly isolated from erysipelas produce slightly larger skin lesions in rabbits than strains from other sources, this property is apparently not attributable to strain specificity since organisms from scarlet fever can after animal passage attain the same degree of skin infectivity as those from erysipelas.

7498 P

Effect of Gonadotropic Hormone Injections upon Hypophyses and Sex-Accessories of Experimental Cryptorchid Rats.

WARREN O. NELSON. (Introduced by M. M. Ellis.)

From the Department of Anatomy, University of Missouri.

The effects of castration upon the anterior hypophysis and accessory sex organs are well known and have been interpreted as resulting from the withdrawal of one or more testicular hormones. Recently, in a study involving an examination of the hypophyses, testes, seminal vesicles, and prostates from 73 cryptorchid and 52 castrate rats it was shown[1] that elevation of the testis into the abdomen (experimental cryptorchidy) at the 40th day of life produced results similar, although retarded, to castration. Changes which definitely indicated a decreasing output of testicular hormone were apparent in the hypophysis regularly at 75 days and in the seminal vesicles at 240 days, post-operation. In the prostates the changes up to 400 days post-operation are barely suggestive of a hormone threshold slightly below that necessary for prostatic maintenance. These results may be correlated with the hormone required for the maintenance of the respective end-organs.[1]

The hypophyses of 360 day cryptorchid rats present a picture similar to that of advanced castration. Cell counts, which have been made regularly, show a marked increase in basophiles, a slight decrease in acidophiles, a more marked decrease in chromophobes, and many castration cells. The testes show no evidence of spermatogenesis and the epithelium of the seminiferous tubules is reduced to Sertoli cells with few if any spermatogonia. Interstitial cells appear to be more numerous, but it is not certain that the increase is more than an apparent one coinciding with tubular decrease. The cells of the seminal vesicle epithelium are low cuboidal to flat and without secretion granules. The prostates are essentially normal in size and cytology. It would seem that the testes of 360-day cryptorchid rats are secreting a small amount of hormone, *i. e.,* enough for prostatic maintenance, but not sufficient to maintain normal seminal vesicles and hypophyses.

Nine 360-day cryptorchids were operated and one testis, one seminal vesicle, and a sample of prostate were removed, weighed, and studied microscopically. The animals were then divided into 3 groups and injected with 50 R.U. Antuitrin S* daily for 10, 20, and 30 days. At the close of the injection periods the animals were sacrificed and, in each instance, the hypophysis, remaining cryptorchid testis, and sex accessories were removed, weighed and studied.

The seminal vesicles removed after injection were approximately 9 times as heavy as those removed for control purposes. Microscopic study showed complete restitution in each instance. The epithelial cells were high columnar with many clearly defined secretion granules. The prostates, too, showed definite evidence of stimulation. These results can be interpreted only as evidence that the treatment had stimulated production of testis hormone.

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[1] Nelson, W. O., *Anat. Rec.*, 1934, **58**, 30.

* Antuitrin S kindly furnished by Dr. Oliver Kamm, Parke, Davis and Company.

The anterior hypophyses presented pictures varying with the duration of treatment. Those from animals treated for 10 days showed a definite decrease in castration cells and a degranulation of the non-vacuolated basophiles. These changes were more pronounced in the 20- and 30-day series. In the latter only a few small castration cells and a very low percentage of normal basophiles were found. Many degranulated basophiles were present. This same effect of Antuitrin S injection upon the basophiles of the normal rat pituitary of both sexes, has been observed by the writer. A similar experiment has been reported recently by Severinghaus.[2]

The testes of the present series of animals have been examined carefully and compared with the testes removed prior to the initiation of treatment. No changes were observed in the epithelium of the seminiferous tubules. Although there appeared to be some effect of the treatment upon the interstitial cells it was neither constant nor distinct enough to be judged significant at this time. The use of more refined cytological procedures might be illuminating in this regard.

A series of 360-day castrates were subjected to the same Antuitrin S treatment. There was no apparent effect on the castration condition of the hypophyses or sex accessories. This result demonstrates that the testis plays an essential intermediary rôle in the changes produced in the pituitary and sex-accessories.

It is apparent from the effect on the sex-accessories that the treatment with gonadotropic hormone stimulated the production of testis hormone by some component of the cryptorchid testis, probably the interstitial cells. It cannot be definitely said that the correction of the castration changes in the pituitary resulted from the same hormone that affected the accessories since it is conceivable that the treatment stimulated the production, by the testis, of some other hormone. However, the evidence is such that it is highly unlikely that the testis secretes a second hormone peculiar to the male gonad. On the other hand, evidence is accumulating which indicates that oestrin is produced in the male either directly by the testis or by a conversion of the male hormone, and that it may be intimately concerned in male reproduction. Furthermore, it is not improbable that oestrin plays a part in the normal control of the anterior hypophysis in the male, a point that we are investigating.

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[2] Severinghaus, A. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 593.

7499 P

Active Constituent of Local Anesthetic Solutions.

JOHN H. GARDNER,* JOSEPH SEMB AND HELEN TREDWAY GRAHAM.

From the Departments of Chemistry and Pharmacology, Washington University, St. Louis.

Such local anesthetics as are salts of weak bases and strong acids—and most local anesthetics come under this chemical classification—have repeatedly been shown to be more effective, particularly for surface anesthesia, anesthesia of isolated nerves, etc., when their solutions have been hydrolyzed to some degree by the addition of alkali. This and other similar evidence led to the theory that the base is the only active constituent of such solutions.[1]

This theory has been put to direct test by comparing the local anesthetic activity of solutions of an anesthetic base alone with that of solutions containing the same concentrations of base plus varying concentrations of the anesthetic salt. The anesthetic activity was measured by the time required to anesthetize to a strong break induction shock the nerve of a sciatic-gastrocnemius preparation from *Rana pipiens;* the time for recovery after removal from the anesthetic solution was also sometimes recorded.

The presence of the salt (hydrochloride) has been found to make no difference in the time required to anesthetize with γ-4-morpholinepropyl benzoate, one of a series of recently synthesized local anesthetics[2] (Table I). However, in all cases, as in this one, the presence of salt results in a shift of pH towards the acid side, and this might alter the sensitivity of the tissue or otherwise modify the

TABLE I.

Frog	Concentration of the anesthetic in mols per liter Base	Salt	pH	Time to anesthesia Min.
1	.004	0	8.1	10
1	.004	.004	6.9	10
2	.004	0	8.1	12
3	.004	0	8.1	8
3	.004	.004	6.9	8
4	.004	.004	6.9	8
5	.002	0	8.1	60
5	.002	.002	6.9	60

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* This work was made possible by a grant to the Department of Chemistry from a fund given to Washington University by the Rockefeller Foundation for research in science.

[1] Trevan, J. W., and Boock, E., *Brit. J. Exp. Path.*, 1927, **8**, 307.

[2] Gardner, J. H., and Haenni, E. O., *J. Am. Chem. Soc.*, 1931, **53**, 2763.

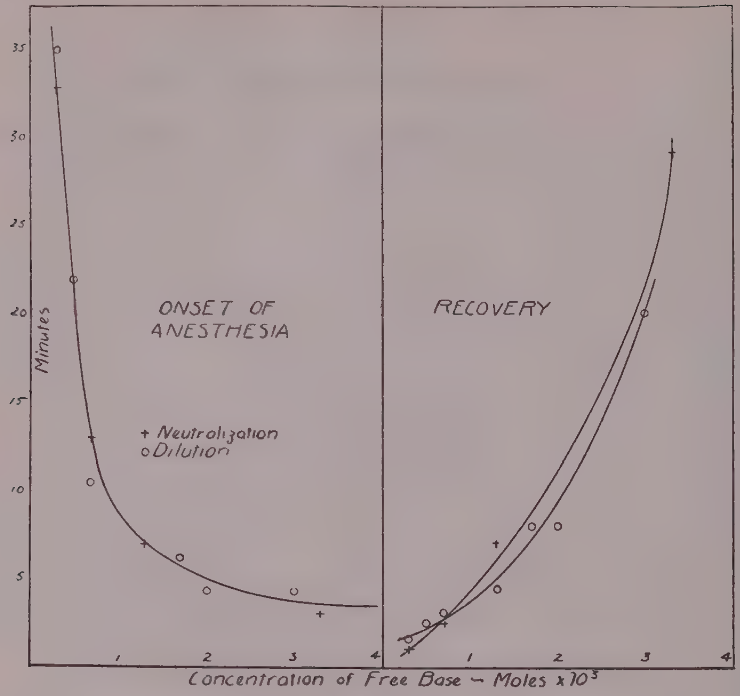

Fig. 1.

effectiveness of the anesthetic in such a way that, for instance in the example given, a total anesthetic concentration of 0.008 mols per liter had the same activity at pH 6.9 as 0.004 mols at pH 8.1.

This possibility is eliminated, however, by the results plotted in Fig. 1. To a portion of 0.004 molar procaine hydrochloride solution, enough sodium hydroxide was added to liberate the base completely, and a series of solutions containing various concentrations of the base was prepared by dilution. Another series of solutions of various concentrations of the base was prepared by neutralizing to varying degrees other portions of the original procaine hydrochloride solution (pH of series, 5.1-9.6). In the figure the 2 series of solutions fall on the same curve when the time to anesthetize is plotted against the concentration of free base, and coincide almost as closely when the time for recovery is used as the measure of anesthetic activity. It is evident that neither the total anesthetic concentration nor the pH but the concentration of free base is the variable in the 2 series determining the anesthetic activity.

7500 P

Cultivation of Mycobacterium Leprae. III.*

M. H. SOULE.

From the Hygienic Laboratory, University of Michigan.

The experiments here recorded were made with the object of confirming in a distant land and with patients of another race our earlier studies[1] on the isolation and cultivation of the leprosy bacillus. A description was given of a slow growing non-chromogenic acid-fast organism isolated from human leprous tissue by incubating the inoculated media in an atmosphere of 40% O_2 and 10% CO_2. There was unequivocal evidence of the proliferation of the germs but it was also true that the quantity was limited. The organisms were removed from the positive tubes and transferred to freshly prepared sterile media; after an incubation period of about 6 weeks the tiny colonies that had developed were subcultured as before. A total of 26 serial subcultures were made but there was no apparent improvement on the part of the cells to assume a saprophytic existence. The culture in the 26th generation showed the same characteristics as at the beginning of the series.

A subsequent communication[2] reported efforts to provide a more suitable substratum. Several of the complex formulae ordinarily employed for the isolation of such species as Johne's bacillus and the tubercle bacillus were prepared and inoculated; in addition amino acids and fresh vegetable tissues were incorporated in several of the usual laboratory media. The results were uniformly disappointing. The overwhelming numbers of organisms in the lesions of the disease and the impoverished growth in test tubes led to the conclusion that some essential food factor was lacking in the nutritive media.

There have been several criticisms directed at the studies carried out in Puerto Rico; the most important of which has been the suggestion, that in making the serial subcultures, tissue debris containing stainable organisms was mechanically transferred from tube to tube, leading to the erroneous conclusion that proliferation had occurred when actually no *in vitro* multiplication had taken

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* These investigations were carried out with the aid of a grant for research made by the Leonard Wood Memorial for the Eradication of Leprosy. The author wishes to express his indebtedness to Dr. J. Fajardo, Director of Health in the Philippines, also to Dr. C. B. Lara, Chief of the Medical Staff of the Culion Leper Colony and his colleagues for their kindness.

[1] Soule, M. H., and McKinley, E. B., *Am. J. Trop. Med.*, 1932, **12**, 1.

[2] Soule, M. H., and McKinley, E. B., *Am. J. Trop. Med.*, 1932, **12**, 441.

place. The appearance of definite colonies, although of limited size, gave assurance that such was not the case, however in planning the protocols for the studies in the Philippines adequate controls were included to obviate this as well as other objections.

The details of the experiments followed in general the technique used in Puerto Rico. Clinical specimens (excised nodule emulsions, subepidermal pus from "lepra reaction" cases and broken down nodules) rich in acid-fast forms were transferred to the surfaces of the media contained in tubes; usually 6 of these were inoculated from each specimen. The tubes were then placed in Novy jars and a special gaseous environment was provided containing 10% CO_2 and 40% O_2. After an incubation period of 4-6 weeks the media were examined macroscopically for the presence of minute colonies and microscopically for acid-fast forms. In every cultural attempt, to control the mere mechanical transfer of stainable cells, there were placed in the jar, tubes which were inoculated with a droplet of the original specimen that had been autoclaved to insure the death of the cells. This method of killing the organisms did not alter their tinctorial properties. When the control tubes were examined microscopically it was possible to find after a careful search a few well formed acid-fast rods in the subcultures up to and including the 6th serial transfer, but not thereafter.

The tubes considered positive at the end of the incubation period were subcultured to similar media, placed in the artificial gaseous environment and the incubation again carried out.

The data are presented in Table I.

TABLE I.
Isolation of Slow Growing Acid-fast Organisms, Supposedly Hansen's Bacillus, from Various Types of Infected Material.

Origin of Specimen	Total No. of Specimens Inoculated	No. of Specimens Giving Positive Cultures
Nodules	20	12
Reaction Pus	16	11
Broken down nodules	6	2
Total	42	25

The colonies appearing on the various media were identical with those previously described, and in each instance the organisms were acid-fast. No other colonial or morphological types were observed in the cultures.

The encouraging results reported by McKinley and Verder[3] in the cultivation of the leprosy bacillus with the aid of minced chick embryo tissue suggested the desirability of similar studies at Culion. Accordingly, the minced tissue suspensions were prepared in Erlenmeyer flasks and subsequently inoculated with fresh infected human tissue rich in acid-fast forms. Twenty-six specimens were studied following the technique given by these workers with the addition of controls inoculated with autoclaved material. In 22 instances there was unquestioned evidence of proliferation.† It was possible to find stainable cells in control flasks but the numbers were negligible. The positive cultures were serially subcultured for 6 transfers in the tissue containing medium without any marked improvement as regards multiplication. At various times, test tubes containing the media of Petragnani or Löwenstein were inoculated with organisms from the tissue culture flasks with the hope that the bacteria might possibly be acclimatized to a saprophytic existence after this long residence away from the human host and grow luxuriantly, but such was not the case. The colonies appearing on the special media developed very slowly and were of the typical tiny variety.

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[3] McKinley, E. B., and Verder, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 659.

The writer cultivated the germ of leprosy from infected tissue according to the original technique of Carrel. The results have been uniformly disappointing in that growth, though positive, was no more luxuriant than that obtained with macerated chick embryo tissue.

The isolation and serial cultivation of a slow-growing nonchromogenic acid-fast organism from human leprosy tissue has been confirmed. The limited multiplication of the germs indicated that the ideal media and environment for their saprophytic existence has not been provided.

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† McKinley and Verder had noted early in their work the similarity of the rod shaped pigment cells of the chick retina with clumps of bacilli and had kindly issued the warning not to be misled by these forms.

7501 P

Bacteriology of Leprosy. IV. Bacteremia.*

M. H. SOULE.

From the Hygienic Laboratory, University of Michigan.

Cultures were made of the blood of 462 patients suffering with advanced leprosy in its various manifestations. Only one sample of 10 cc. was taken from each patient and a separate sterile syringe was used in each instance. The skin over the arm vein was thoroughly scrubbed with alcohol and tincture of iodine was applied and allowed to dry 2 min. before the insertion of the needle. The contents of the syringe were transferred directly into the culture or centrifuge tubes.

Two hundred specimens were cultured by the technique of Löwenstein.[1] The blood was centrifugated at once, the serum withdrawn with Pasteur bulb pipettes, the red corpuscles dissolved by the addition of sterile distilled water until the hemoglobin was entirely removed. The precipitate was then transferred to the surface of Löwenstein's medium which had been distributed and sterilized in Blake bottles of 250 cc. capacity. After inoculation, the bottles were sealed with wax and placed in the incubator at 37° for 6-8 weeks. At the end of this interval the flasks were removed from the incubator and the surface of the medium carefully examined macroscopically for the presence of colonies and later microscopic studies were made of stained smears of the water of condensation. No growth was noted.

Two hundred samples of blood were transferred directly into 30x250 mm. tubes, each containing 50 cc. of cooked meat beef heart broth prepared by the addition of heart infusion broth to pieces of cardiac muscle saved from the infusion. The tubes were placed in the incubator and at weekly intervals for a period of 8 weeks a loopful of the broth suspension was streaked over the surface of NNN medium in test tubes. No growth appeared on this medium after incubation.

Sixty-two specimens of blood were allowed to clot in 50 cc. sterile plugged centrifuge tubes. The clots were separated from the walls of the tubes with a sterile wire and subsequently centrifugated at 500 R.P.M. for 10 minutes. The serum was removed with Pasteur bulb pipettes and 50 cc. of sterile glycerol infusion broth were added to each clot. The centrifuge tubes containing the clot and medium were then placed in the incubator. The tubes were removed and the medium examined daily for evidence of growth. At weekly intervals, for 6 weeks, a loopful of the broth was streaked over the surface of NNN medium and subsequently incubated. No growth was obtained.

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* These studies were carried out at the Culion Leper Colony, Culion, Palawan, P. I., with the aid of a grant for research made by the Leonard Wood Memorial for the Eradication of Leprosy.

[1] Löwenstein, E., *Inter. J. Leprosy*, 1933, **1**, 39.

The negative results obtained as a result of this rather large series of experiments are rather hard to interpret in the light of many recent studies[2] on the isolation of organisms from the blood stream in health and disease. It is possible that the therapeutic measures instituted against the Hansen infection, namely, the weekly injection of 4-5 cc. of the iodized ethyl esters of *Hydnocarpus wightiana* oil, may have had a direct influence on the incidence of blood stream invasion. It is also true that these studies were made during the rainy season so that the possibility of air borne contamination was at a minimum.

7502 P

Effect of Testicle Extract on Primary Tuberculous Infection and Reinfection in Guinea Pigs.

ROBERT M. THOMAS AND F. DURAN-REYNALS. (Introduced by Florence R. Sabin.)

From the Laboratories of the Rockefeller Institute for Medical Research.

1. *Effect on Primary Infection.* Testicle extract greatly increased the extent of the local lesions resulting from the intradermal or subcutaneous injection of virulent human tubercle bacilli into guinea pigs. When compared with lesions resulting from the injection of an equal number of tubercle bacilli suspended in salt solution, the increase was estimated at from 10 to 15 fold. The testicle extract was prepared by mincing bull testicle in 2 volumes of physiological salt solution, straining the material through cloth and finally filtering through a Berkefeld V candle. Kept at ice-box temperature, the extract maintains its activity for a long period of time. Two groups of 6 guinea pigs were used in the first experiment, in which the injection was made intradermally. One group

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[2] Callow, Bessie R., *J. Inf. Dis.*, 1933, **52**, 779.

was injected with a mixture of 1.0 cc. of testicle extract and 1.0 cc. of a suspension of H-37 strain of human tubercle bacilli in salt solution, containing 0.1 mg. of organisms, moist weight. The other group of 6 guinea pigs was injected with 2.0 cc. of a suspension of tubercle bacilli in salt solution, containing 0.1 mg. of organisms. The lesions resulting from these intradermal infections in the case of the testicle extract group were very large, spreading over the entire flank, from 5 to 6 cm. in width and from 7 to 8 cm. in length. The controls showed small nodules which ulcerated and drained, at the most 1.5 to 2.0 cm. in diameter.

A second experiment was performed using 2 groups of 10 guinea pigs, in which the injections were made subcutaneously in the groin. The dosage and quantities of testicle extract were the same as in the first experiment above.

Both experiments were terminated from 60 to 70 days after infection. The animals that had been infected with the addition of testicle extract showed, besides a more extensive primary infection, a much greater amount of generalized tuberculosis. The increase in the extent of visceral lesions was apparently due to the greater extent of the primary lesion, resulting from the spreading of the injected organisms, rather than to a systemic effect of the testicle extract, since in experiments which are still being carried on, there is evidence that the parenteral injection of testicle extract after infection has been established does not cause any appreciable increase in the amount of tuberculosis which develops.

2. *Effect on Tuberculin Reaction.* Testicle extract was added to solutions of tuberculo-protein MA-100 (Mulford) in the proportion of 0.7 cc. of testicle extract to 0.3 cc. of a solution containing from 0.25 to 3.0 mg. of tuberculo-protein. Injected intradermally into tuberculous guinea pigs, the skin reactions were spread over a large area, 4.5 cm. in diameter, in comparison with the smaller but more intense reactions resulting from the injection of a like amount of tuberculo-protein without the testicle extract. The reactions, while increased in extent, due to the spreading factor in testicle extract, were less intense and of much shorter duration than in the controls.

3. *Effect on Reinfection.* Guinea pigs that had been infected subcutaneously with 0.01 mg. of H-37 tubercle bacilli from 4 to 6 weeks previously, were reinfected with 0.1 mg. of H-37 bacilli, suspended in 1.0 cc. of salt solution and 1.0 cc. of testicle extract. Another group of guinea pigs that had been infected at the same

time were reinfected with 0.1 mg. of H-37 bacilli suspended in 2.0 cc. of salt solution. A total of 36 animals were reinfected, 18 with the addition of testicle extract and 18 without. The lesions in the animals reinfected with testicle extract were 4 or 5 times larger than those of the controls and were characterized by a progressive induration which, after 2 to 3 weeks, resulted in the formation of a hard, dry, necrotic area which healed very slowly from the edges. Sloughing of the surface of the necrotic mass occurred in some instances, leaving a shallow indolent ulcer. At no time did abscess formation occur, nor did the lesions discharge any material.

An interesting and striking result of such reinfection was that the progress of the disease in the group with testicle extract was greatly retarded in comparison with the controls, and with the lesions in untreated controls of primary infections. The animals were sacrificed from 4 to 6 weeks after reinfection, and in 3 separate experiments the same condition and results prevailed.

The animals reinfected with testicle extract showed from 40 to 60% less tuberculosis than did the controls or untreated controls of primary infection.

In each of the 3 experiments, also, the ulcers of primary infection showed healing more frequently in the animals reinfected with testicle extract. Further experiments on the nature of this effect are in progress.*

No benefit was found to derive from the reinfection of tuberculous guinea pigs with BCG, with or without testicle extract, nor from the repeated injection of heat-killed human tubercle bacilli, with or without testicle extract.

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* In an additional experiment recently completed, 5 guinea pigs with far advanced tuberculosis were reinfected by intracutaneous injections of living H37 bacilli with testicle extract. For some reason probably connected with the far advanced state of the disease the resultant skin lesions were practically of the same size and appearance as those which occurred in four animals reinfected at the same time with a saline suspension of bacilli. Also there was no appreciable difference in the extent of the general lesions in the two groups, when sacrificed 34 days later.

7503 P

A Suggested Test for Functional Cortical Adrenal Tumor.

ROBERT T. FRANK.

From the Laboratories, Mount Sinai Hospital, New York City.

A clinical syndrome characterized by facial and trunk obesity, hirsutism, pig eyes, either persistently high blood pressure or attacks of hypertension, amenorrhea or menstrual irregularity, pinkish skin striae, rarefaction in the bones, polycythemia, acrocyanosis of extremities and susceptibility to infection, has attracted increasing attention, particularly since Cushing described basophilic adenoma as a probable cause.[1] Previously a number of these cases had been found due to cortical adenoma or adrenal tumor.[2] It might readily be conceived, as suggested by Cushing, that a pituitary overfunction, perhaps limited to the basophilic type of cells, might secondarily produce cortical adrenal changes.

A typical case, diagnosed as "basophilic adenoma" was under my observation in my clinic and laboratory for 1½ years during which time thorough hormonal studies were performed with extremely striking findings. This patient died suddenly (on the service of Dr. B. S. Oppenheimer) in consequence of an erysipelas secondary to an acute middle ear infection (unoperated), permitting a full autopsy. At autopsy the pituitary was found normal (serial sections, Dr. Joseph Globus), the basophilic elements proving diminished in number. A large carcinoma of the adrenal cortex was found. The case will be described in detail elsewhere. At the moment, the hormonal findings alone will be discussed.

Hormonal examination, extending over a period of 4 weeks, showed negative pregnancy tests, no increase in the prepituitary and female sex hormones circulating in the blood.[3] On the other hand, the excretion of female sex hormone in the urine was at times tremendously increased, to a degree seen normally only in pregnancy (13,000 Mouse Units per liter). Fig. 1. During what corresponded to one cycle, this patient excreted more than 57,000 M.U. of female sex hormone. It was repeatedly possible to obtain a mouse reaction with as little as a total of 0.075 cc. of undiluted urine 48 hours after injection into the castrated mouse.

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[1] Cushing, Harvey, Papers Relating to the Pituitary Body, Hypothalamus and parasympathetic System, Charles C. Thomas, Springfield, Illinois, 1932, 113 *et seq.*

[2] Thumin, *Berlin. Kl. Wchensch.*, 1909, No. 3.

[3] Frank, R. T., *J. Am. Med. Assn.*, 1931, **97**, 1852; Frank, R. T., Goldberger, M. A., and Spielman, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 999.

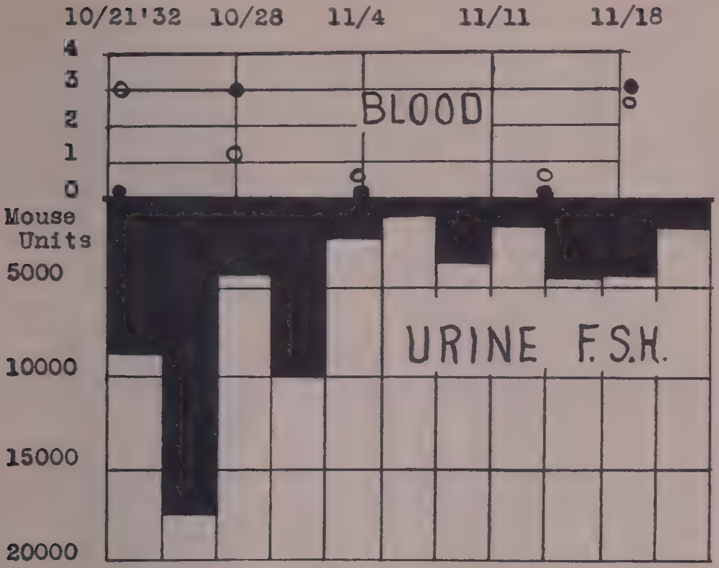

FIG. 1.

Through the courtesy of Dr. John Wyckoff of Bellevue Hospital and Dr. Douglas Simmers of this hospital, I was able to study another case. This patient presented all the cardinal symptoms previously mentioned, and was seen one week before death.

The undiluted urine of this patient likewise gave a strong female sex hormone reaction in quantities of 0.2 cc. (5000 M.U.L.).

At autopsy of the Bellevue patient, no basophilic adenoma of the pituitary was encountered. A large carcinoma of the adrenal cortex with metastases in the liver was found. Extracts made from the primary and secondary tumor in the liver showed twice as much female sex hormone in the extracts, as in control tissues such as the spleen.

Since these findings were obtained, I have had occasion, through the courtesy of various colleagues, to test a number of other patients who have shown at least some of the symptoms ascribed to the above mentioned syndrome, without encountering the same high urinary findings of female sex hormone, as in the 2 cases of undoubted cortical tumor. As these patients have not been operated upon and are still alive, they do not enable me to draw further conclusions.

Because of the rarity of the "basophilic pituitary adenoma" and of cortical tumors of the adrenal, I feel justified in drawing the attention of the profession to the hormonal findings obtained in these 2 clearcut cases, with the hope that this test (high female sex hormone excretion in the absence of positive pregnancy reaction) may serve as a means of recognizing adrenal cortical tumors at an early and operable stage. Perhaps this test will also serve in differentiating them from the "basophilic pituitary adenomas" if further observations prove that their presence does not produce the same overexcretion.*

7504 C

Importance of the Spleen as a Reservoir for Erythrocytes.

E. VON HAAM.

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One of the modern conceptions of the physiology of the spleen is to regard this organ in human beings and in animals as a reservoir which is able under different conditions to pour red blood cells into the circulation (Landa[1]). The discrepancy of the results obtained by various authors (Landa and Haam, Radosaljevic and Sekulic, Testoni and others[2]) concerning the appearance or nonappearance of adrenalin polycythemia in splenectomized animals makes the supposition acceptable, that other organs or organ systems substitute sooner or later the erythrocyte-storing function of the spleen.

The methods of our experiments were simple. Three dogs and 3 puppies were splenectomized and kept under observation for 18 months following the operation. Before the splenectomy the degree of the adrenalin polycythemia in the peripheral bloodstream was determined in 2 consecutive examinations, and the same test was repeated shortly after the animals had recovered from the splenectomy and thereafter at regular intervals. Care was taken that the animals were kept under healthy physiological conditions with plenty of freedom and exercise. Two of the splenectomized puppies died 3 and 5 months respectively after the splenectomy, of intercurrent diseases. Because of their illness and subsequent death the results of their tests have been discarded entirely from our charts. The other animals remained healthy and are being kept for further observation. The postoperative anemia, which could be observed in a mild degree in all of our operated dogs, disappeared after 2 or 3 months.

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* Since this paper was handed in for publication, 10 further cases which showed at least some of the symptoms of so-called basophilic adenoma—all of them hirsutes, 4 with increased blood pressure, 3 with striae, were examined with negative results. These included 3 cases obtained through the kindness of Drs. Wilder and Snell of the Mayo Clinic, 2 in whom cortical adenomas of the adrenal had been removed some time previously, the patients remaining well; and one in whom cortical hyperplasia was found at operation, the urine obtained just before operation, all proved negative. These findings make me all the more anxious to have the profession apply the test in order to determine whether the high female sex hormone titer in the urine is limited to cases with metastases, similar to the large amount found in testicular tumors not containing chorionepithelioma, when general dissemination occurs.

[1] Landa, E., Physiologie der Milz, Urban & Schwarzenberg, Wien, 1933.

[2] Landa, E., and Haam, E., *Z. f. exp. Med.*, 1932, **80**, 640; Radosaljevic and Sekulic, *Wien. Arch. f. inn. Med.*, 1930, **20**, 81; Testoni, A., *Arch. internat. Pharmacodyn.*, 1930, **37**, 1.

The results of our experiments are reported at this time because no changes in the curve of the peripheral hematocrit values following adrenalin could be observed for the last 6 months. The technic of the test for the acute polycythemia in the peripheral blood circulation following adrenalin injection was the same as reported in previous experiments. Each test was made under the same conditions and with chloralose as the anesthetic. Since the results of the experiments on our 4 surviving dogs were rather uniform, as can be seen from Table I, the curves of Dog W may be taken as a good example for the entire experiment.

It can be seen that the highest rise in the hematocrit value following adrenalin injection could be observed before the operation. Shortly after splenectomy adrenalin was not able to produce polycythemia. Eighteen months after the operation, however, the hematocrit value of the peripheral blood showed a distinct increase after the intravenous injection of adrenalin.

The results obtained on all 4 dogs during the 18 months of observation are summarized in Table I.

In all 4 dogs the presence of acute polycythemia following adrenalin injection could be demonstrated after splenectomy. In some

TABLE I

Maximal Increase of the Hematocrit Value after Adrenalin Injection

Dog	Before Splenectomy	Months after Splenectomy ½	1	2	3	[illegible]	[illegible]	12	[illegible]	18
B	15.6	2.4	[illegible]	1.4	[illegible]	[illegible]	[illegible]	[illegible]	7.2	[illegible]
C	8.2	[illegible]	1.8	2.8	[illegible]	4.7	[illegible]	[illegible]	[illegible]	[illegible]
W	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
P2	[illegible]	[illegible]	[illegible]	4.8	[illegible]	8.4	[illegible]	[illegible]	[illegible]	[illegible]

dogs this could be shown as early as 5 months after the spleen had been removed; in others the polycythemia appeared later. In none of the animals did it reach the same height as observed before the operation. The highest value of adrenalin polycythemia could be observed in the splenectomized puppy (P_2).

Conclusions. Repeated examinations of the hematocrit value of the peripheric blood following the intravenous administration of adrenalin in 4 dogs before and for a period of 18 months following splenectomy could demonstrate that (1) the acute polycythemia following adrenalin which is regularly observed in the normal dog disappears after splenectomy; (2) after that period the polycythemia returns but does not reach the same degree as in the normal dog. These results suggest a slow substitution of the erythrocyte-storing function of the spleen by another capillary system.

7505 C

Conditions of the Halo Formation Around Subtilis Colonies and Growth of Halo Transplants.

OCTAVIA PEIRSON AND LOUIS DIENES.

From the Department of Pathology and Bacteriology, Massachusetts General Hospital, Boston.

An interesting phenomenon was described[1] which certain *B. subtilis* strains show when grown on nutrient agar containing a small amount of saccharose. The amorphous, stringy material which is present in abundance in the cultures as an intercellular substance spreads out from the colonies and forms a wide halo around them. In the halo bacteria are not visible and transplants of it do not grow on the usual media. Transplanted on agar plates containing saccharose the halo substance grows in tiny transparent colonies. The observations described in the following note[2] furnish convincing evidence that these growth phenomena are caused by the growth of living elements entirely different in morphology from the usual forms of bacteria.

The observations here described concern the conditions under which the halo develops and which determine the abundance of growth in the transplants.

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[1] Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1205.

[2] Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1211.

The formation of the halo depends on the addition of saccharose to the nutrient agar. Usually as small an amount as 0.02% is sufficient and about 0.1% gives the best results. It is probable that the saccharose itself is effective and not an impurity in the saccharose preparation; because, by fractionation of saccharose with alcohol, no fractions were obtained which had increased or decreased potency. Other sugars have no effect on the halo formation. The formation of the halo depends largely upon the condition of the surface of the plate. The production of the halo can be greatly improved by rubbing the surface of the agar plate lightly with a glass rod. The different batches of agar vary very slightly and the addition of different animal proteins, fresh plant tissue, cystein, etc., produced no effect. When the pH is varied between 6 and 8.5 there are no marked changes.

For the closer study of the conditions upon which the growth of the halo substance depends we started with an agar medium containing no nutrient substances besides saccharose. The growth of our strains, as was already indicated in another place, is very characteristic on this medium. No halo is formed but the bacterium colonies are surrounded by a haziness which under the microscope consists of tiny colonies. These tiny colonies vary in size from a few hundredths to a few tenths of a millimeter and are situated mostly deep in the agar. In the tiny colonies no bacteria are visible. If a small piece of agar containing these colonies is cut out and transferred to another agar plate a similar growth is produced without bacterial growth. Transplants from the halo formed on nutrient agar plates also grow similarly on non-nutrient agar plates which shows that the formation of haziness is an analogous phenomenon to the halo formation. Haziness in the non-nutrient agar plates is produced more often and more constantly by bacteria than a typical halo.

Various substances were added to the non-nutrient agar and the effects on the formation of the tiny colonies were studied. In all cases where the medium contained vegetable or animal substances although the growth of bacteria was markedly increased the formation of the tiny colonies was inhibited. If the substances were added in very small concentrations the growth of tiny colonies reappeared, but their growth was never improved by these additions. The addition of various inorganic salts was either detrimental or without effect. Only increasing the concentration of the agar from 2% to 4 or 6% materially improved the growth of the tiny colonies. Halo was never produced in these experiments. CO_2 and absence of O_2 did not affect the development of the tiny colonies.

It was evident from these observations that the agar itself contains the substances which make the development of the tiny colonies possible. The agar used in this experiment was prepared by autoclaving commercial agar in tap water and pouring off the clear part of the agar after sedimentation. The medium always contained impurities including dead bacteria. The next step in the study was to free the agar from the impurities by filtration. A weak solution (0.5%) of agar was first passed through paper pulp, then through a Berkefeld filter and concentrated to 4% on the water bath. A perfectly clear agar was obtained. After the addition of saccharose to this agar the growth of the bacteria was very poor—in contrast to the abundant growth on the non-filtered plates—and the tiny colonies did not develop. By adding different kinds of nutrient substances the growth of the bacteria was greatly increased but neither the tiny colonies nor the halo was obtained. Filtering the agar simply through paper also considerably decreases the growth of tiny colonies.

Since the tiny colonies in the non-nutrient agar tend to grow around the impurities present in the agar we tried to add various kinds of non-soluble material to the filtered agar. Tiny bits of meat thoroughly extracted by water, starch, $CaCO_3$, iron oxide, carbon obtained by heating saccharose, all were without effect. Impressive effect was obtained by the addition of autoclaved earth and infusorial earth suspensions. The whole medium was full of tiny colonies growing around the particles. Filtered or thoroughly centrifuged earth extract did not have this effect. The ignition of earth or the extraction of infusorial earth with HCl followed by ignition did not decrease their effectiveness. The addition of ground glass was also effective. It is probable, however, that the filtration of the agar affects the growth of tiny colonies not only by the elimination of silicon dust but also by the elimination of other substances.

With the help of infusorial earth the surface growth of the halo colonies can be made more abundant. The surface of a non-nutrient agar plate is sprinkled lightly with sterile infusorial earth and the transplant from the halo is made on this area. Without infusorial earth the growth is poor and only the haziness developing in the agar is visible without a hand glass. The soft transparent growth obtained with the help of infusorial earth may be as abundant as, for instance, a confluent growth of pneumococcus. The second transplant made in this way is also usually abundant. The third less so, and in the fourth only a few colonies develop.

The negative or depressing effect of all kinds of nutrient material and the stimulating effect of earth on the expansion of the halo on non-nutrient agar plates are observations which probably will be of interest for the further study of the halo organisms and their relationship to the bacteria. Our strains were obtained usually from contaminated blood cultures and originated probably from dust. Numerous colonies showing halo formation grew from dust collected under the roof of the laboratory building. Adding earth to the agar probably makes the medium more similar to the natural habitat of our strains and the addition of nutrient material less similar. It seems rather probable from this observation that the peculiar process which we observed is a regular process in the natural life of our strains, which is usually not apparent in artificial growth but under the influence of saccharose and other appropriate conditions will be noticeable. The halo formation on nutrient agar is a peculiar case of growth and expansion of the halo substance, the conditions of which are entirely different from the conditions which regulate the extension of the halo substance into the medium of non-nutrient plates.

7506 P

Morphologic Elements in the Halo of Subtilis Colonies.*

L. DIENES.

From the Department of Pathology and Bacteriology, Massachusetts General Hospital, Boston.

The most interesting question in connection with the phenomena which are the subject of the previous note[1] is whether they are life phenomena produced by the growth of living organisms or whether they represent only a peculiar combination of diffusion phenomena and enzymatic action. The number of successive transfers in which the halo substance so far was grown is not sufficiently high to exclude the supposition that the mucoid substances are synthesized in the medium by an enzyme excreted from the bacteria. The observations which will be described in this note show that the halo consists of organized elements and is not an amorphous substance.

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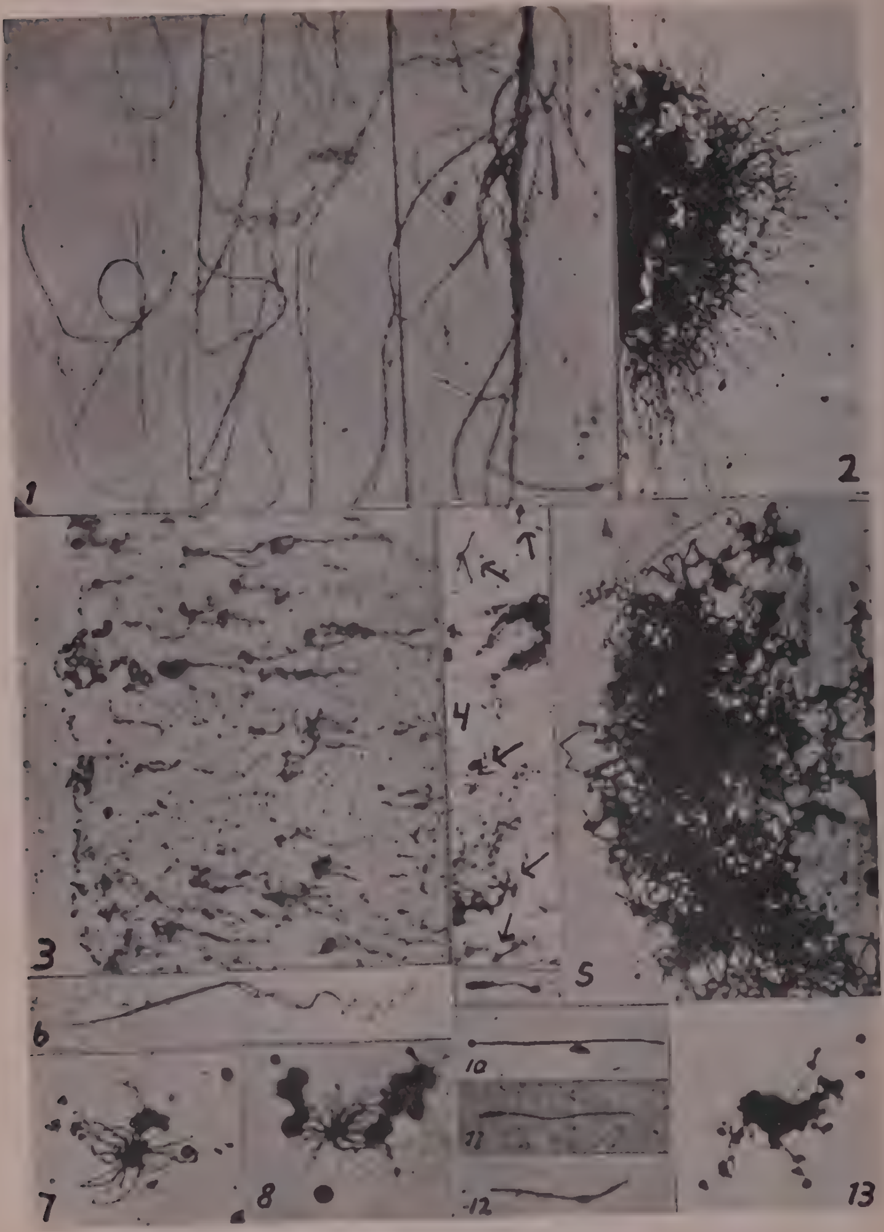
* The publication of the figures was made possible by a grant from the DeLamar Research Fund of Harvard University.

[1] Peirson, O., and Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1208.



The magnification in all figures is 1:1000.
The preparations were stained by Zettnow's flagellar staining method.
FIG. 1. Long fine filaments from halo.
FIG. 2. Short fine filaments from halo transplant.

These observations furnish definite evidence for the living nature of the halo.

Direct examination of the halo under the microscope and examination of preparations stained with crystal violet reveals no formed elements in the halo. If a small piece of agar containing the deep colonies of halo is cut out and stained with crystal violet the colonies remain unstained in the deeply stained agar. These colonies often contain stained granules and sometimes fine bacillary forms but these can not be distinguished from the impurities present in the agar. Examined with dark field illumination the halo consists of a great number of faintly visible granules. The elements which build up the halo can be seen best in preparations stained by flagellar staining methods. For a long time our attempts to stain the halo elements with these methods remained unsuccessful and only after much experimentation were successful preparations obtained.

The elements found in the preparations show an extreme polymorphism. There are larger elements which consist of a straight or bizarre shaped filament which is often pointed or fusiform. They may run out into a fine filament or end in a coccus-like body. These filaments may form large interwoven masses. They are not stained with crystal violet and dark background preparations show that they are much thinner than bacteria. Another larger element is represented by coccus-like bodies which form sometimes with the filament asteroid forms. Sometimes in the halo transplants definite bacillary or coccus forms are found which are faintly stained with crystal violet.

Besides the larger elements bundles of very fine seemingly rigid filaments are found rarely and the mass of the halo substance is built up of fine short filaments or of tiny granules. The staining of the short fine filaments and granules is usually unsatisfactory and their differentiation from artefacts in the preparation is not always clear. The larger forms are visible with dark field methods and their form and arrangement, like the arrangement of the long fine filaments, is so characteristic that no doubt can remain that they represent actual structures.

The larger elements of the halo and halo transplants, though in the latter definite bacterial forms are rarely found, differ in essential

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FIG. 3. Short fine filaments from halo, also a spore-like body in the right lower corner of the figure.

FIG. 4. Asteroid forms from halo.

FIG. 5. Large mass of thick filaments from halo transplant.

FIG. 6. A thick filament from halo running out into fine filaments.

FIGS. 7 and 8. Asteroid forms from halo.

FIGS. 9, 10 and 11. Thick filaments from halo transplants, 12 from halo.

FIG. 13. Filaments and coccus-like bodies from halo transplant.

points from the usual forms of bacteria. First of all they do not reproduce bacterial growth while the bacteria grow profusely in our media. They are extremely pleomorphic, very fragile, and remain unstained by crystal violet. In the halo the larger elements are only sparsely present and as the transplants give rise to thousands of tiny colonies the growth must originate from very small forms. It seems probable that the elements of the halo originate from the bacteria as from a heated spore emulsion all spores reproduce the halo. None of the filamentous structures in the halo correspond closely to the filaments which were described in the bacterium cultures.[2]

It is interesting to note that the elements of the halo which are so different from the bacteria resemble closely the elements from which the cultures of the virus of pleuropneumonia bovis are built up.

7507

Hereditary Variations in Litter-Size of Rabbits.

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From the Laboratories of The Rockefeller Institute for Medical Research.

An analysis of 569 pregnancies occurring in our breeding colony during the 5-year period from 1929 to 1933 has indicated the presence of wide variations in the mean gestation periods of different breeds.[1] These differences were attributed to hereditary factors. The present report is concerned with an analysis* of the size of the litters resulting from these 569 pregnancies, the particular purpose being to ascertain whether breed had any influence on litter-size.

The pregnancies were the result of matings made in all months with the exception of July and August. Eleven breeds consisting of 10 standard bred strains and one intensely inbred line of albinos which may be considered as a breed or family are represented. The mean litter-size ranged from 3.92 for Polish rabbits to 7.14 for the Beveren and Flemish breeds.

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[2] Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 388.

[1] Rosahn, P. D., Greene, H. S. N., and Hu, C. K., *Science*, 1934, **79**, 526.

* The symbols employed below are defined as follows; Var. = Variance; F = the ratio of the larger to the smaller variance; n = number of observations; M_n = mean of n observations; D = difference between two means; t = the ratio of the difference to its standard error; P = probability. For a description of the methods of analysis see Fisher, R. A., Statistical Methods for Research Workers, Oliver and Boyd, London, 1932, and Snedecor, G. W., Calculation and Interpretation of Analysis of Variance and Covariance, Collegiate Press, Inc., Ames, Iowa, 1934.

It was found that the variance between breeds was significantly greater than the variance within breeds (Var. between means of breeds = 40.43; Var. within breeds = 4.67; F = 8.65; P = 0.01—, significant). The demonstration of heterogeneity between breeds is interpreted as indicating that with respect to litter-size, each breed represented a homogeneous, normally distributed population. Certain environmental factors which might account for the breed differences were considered.

All animals received the same diet and were housed indoors under uniform conditions. Seasonal factors at the time of mating did not influence the litter-size, since a significant difference was not noted between the mean values calculated from all matings in the 2 intervals from March to October, and from November to February (March to October: $n = 295$, $M_n = 5.18 \pm 0.14$; November to February: $n = 274$, $M_n = 5.47 \pm 0.14$, $D = 0.29 \pm 0.19$, $t = 1.5$, not significant). Moreover, the variance within 2 months intervals was larger than the variance between 2 months intervals, although the difference was not significant (Var. between means of 2 months classes = 11.53; Var. within 2 months classes = 5.30; F = 2.18, not significant). With respect to season therefore, the population appeared to be homogeneous.

The mean age of the doe at the time of mating ranged from 9.96 months for the American Blue to 14.75 months for the Chinchilla. The method of analysis of variance gave significant evidence that so far as age of the doe at the time of mating was concerned, the whole set of values represented a homogeneous population. (Var. between means of breeds = 5.91, Var. within breeds = 45.64, F = 7.72, P = 0.01—, significant). There was no correlation between the breed mean litter-size and age of the doe at the time of mating ($r_{xy} \doteq —0.185$, not significant).

Body weight appeared to have some influence on the litter-size. In general the lighter breeds such as the Polish and Himalayan had small litters, and the heavier breeds such as the Flemish and Beveren had large litters. When the breeds were divided into 2 classes according to weight, the litter-size of the heavier group was significantly greater than that for the lighter group ($D = 0.96 \pm 0.25$, $t = 3.9$, $P = 0.01$—, significant).

The environmental factors of housing, feeding, season and age did not appear to account for the observed heterogeneity between breeds. It was concluded that unless some unknown environmental condition or conditions were operative, the differences in litter-size of the different breeds were largely dependent on hereditary factors.

7508 C

Changes in Caudal Bones of the Rat as an Index of Ossification.*

N. B. GUERRANT AND R. ADAMS DUTCHER.

From Pennsylvania State College.

The conventional method of determining the rachitic condition of a rat by either the X-ray or the "line test" method has been restricted primarily to an examination of the longer bones of the rat's body, especially the proximal end of the tibia or distal end of the femur. While a study of the structural changes in such bones furnishes a reliable index of the degree of rickets manifested by the animal in question, a comparison of X-ray photographs of the whole body of such animals indicated that other bones of the skeletal framework might offer some advantages over the tibia and the femur in this respect. This appeared to be especially true of the bones of the tail. This particular body structure appeared to offer a series of provisional zones of calcification which could be studied by either the X-ray or the "line test" method. In fact the tail of the rat had been found to be both more easily and more effectively X-rayed than was the leg and, in addition, it afforded possibilities of removing portions of the bony segments for "line test" during the course of the experimental period, without serious consequence to the health and well-being of the experimental animal.

In connection with another investigation being carried out in this laboratory in which a large number of rachitic animals were being involved, a comparison was made of the X-ray and the "line test" findings, where both the tibia and the caudal bones were considered. A general idea of the comparative results obtained may be had by observing Plates 1 and 2, which were obtained from X-rays and "line test" photographs of the bones of 6 typical animals that represented various stages of ossification.

Animals 0 to 4 inclusive were placed on a rickets-producing diet when 21 days of age. At this time their weights ranged between 40

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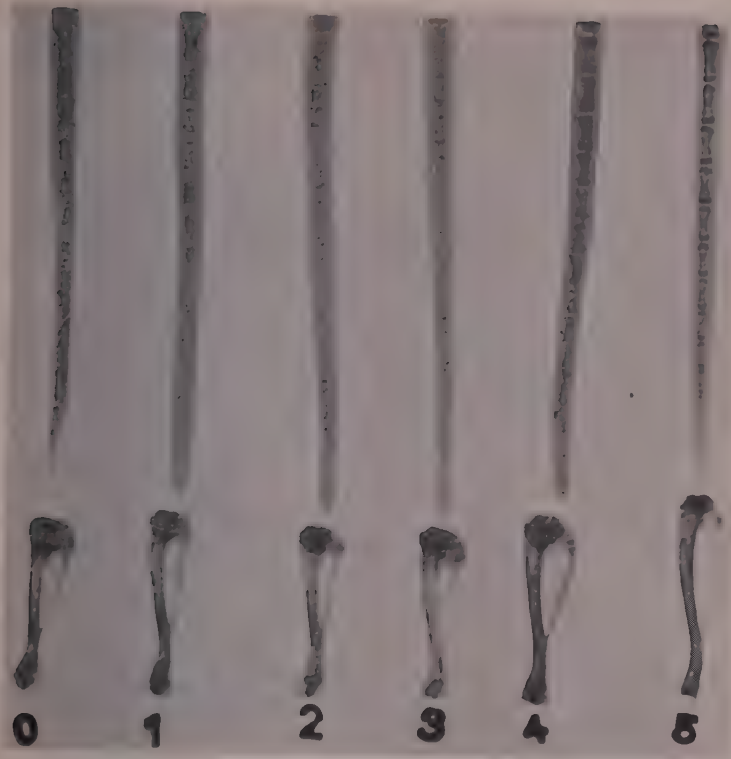

PLATE 1, showing X-ray photographs of both the tibiae and the tails of 6 typical animals, each representing a different stage of ossification.

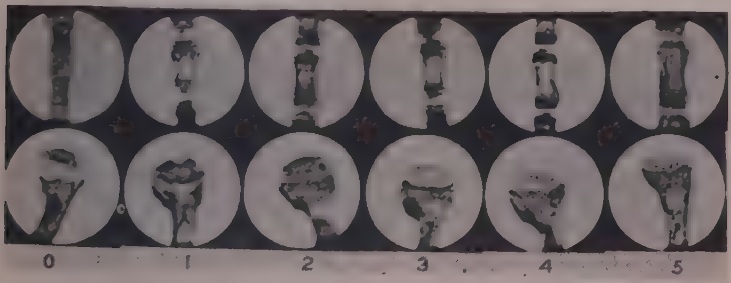

PLATE 2, showing photographs of "line tests" on a tibia and a section of the caudal bones taken from each of the 6 animals considered in Plate 1.

and 46 gm. After being on the rachitogenic diet for 21 days, animal 0 was killed as a negative control, while the 4 remaining animals received one unit (A.D.M.A.) of vitamin D daily as a supplement to the rachitogenic diet. Animal 1 was killed on the 31st day.

animal 2 on the 35th day, animal 3 on the 38th day and animal 4 on the 42nd day. Animal 5 was taken directly from the breeding colony and was 54 days of age when killed.

The tibia and the tail were removed from the dead animal and preserved in a 50% alcohol-water solution until the desired photographs could be made. For the X-ray photographs, the 6 tibiae and the 6 tails were arranged above a single X-ray photographic plate and X-rayed simultaneously. For the "line test" photographs, the bones were prepared in the usual manner and individual photographs were taken.

Through such photographs and other observations, we are led to believe that both X-ray and "line test" made of the caudal bones of the rat are reliable indices of the degree of ossification. The caudal bones appear to offer some advantages over the tibia or the femur in certain respects, but do require greater pains in the preparation for "line testing" than does either the tibia or the femur. Some of these advantages are: (a) the caudal bones are both more easily and more effectively X-rayed, (b) the caudal bones offer a series of zones of calcification instead of a single zone, and (c) the caudal bones offer possibilities of removing segments for "line test" during the progress of the experiment. While such advantages may be important considerations in certain phases of research, it is not suggested that the caudal bones replace the tibia or the femur in the conventional method of determining the state of ossification.

7509 C

Formation of Bone by Periosteum After Experimental Infarction by Embolism of Femur in Rabbits.

G. H. KISTLER. (Introduced by A. D. Keller.)

From the Department of Physiology and Pharmacology, University of Alabama Medical School, and the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago.

Some authors ascribe osteogenic potentialities to the periosteum and feel that it is important for normal nutrition of bone and for repair of injuries to the cortex. Others have observed no nutritional disturbance or decrease in bone regeneration in the absence of periosteum or after experimental fractures, partial resections and drill holes. There is also no uniformity among observations made after transplanting this tissue. Failure of bone formation by the periosteum does not demonstrate that it may not occur under proper conditions. It, therefore, seems desirable to accumulate more data on periosteal osteogenesis and to devise better methods for studying changes in bone.

Infarcts have been produced in the femur of young and adult rabbits by injecting through its nutrient arteries a particulate suspension of charcoal in 5% gum acacia. Interference with the blood supply to the femur by interrupting the vessels outside the cortex did not, however, produce noteworthy changes except in very young rabbits. The bones were removed for examination from 20 hours to 150 days after operation, fixed in 10% formalin and decalcified by 4% nitric acid. Histologic sections were then cut from celloidin blocks and stained with hematoxylin and eosin.

The charcoal emboli produced marked necrosis of the medullary tissues and inner one-fourth to one-half of the cortex. This was followed by the formation of a wide layer of compact new bone outside the cortex that seemed to envelop the infarcted portion of the shaft and it was always well delimited from the pre-existing living bone by a narrow straight line of demarcation. Its thickness varied from a few layers of bone cells to that of the original cortex, and the outer edge was irregular. More marked osteogenesis and revascularization of necrotic bone occurred at the attachment of muscles and tendons. In some regions there was necrosis of the entire thickness of the cortex without new bone formation as if some of the soft tissues about the femur had been infarcted inadvertently. The structure of the new periosteal bone was essentially like that of the cortex but its haversian systems were more irregularly arranged and the bone cells varied in size and shape. The tissues were also slightly less compact due to more abundant and larger canaliculi and blood vessels. Within the medullary canal near the ends of the infarcted regions, new endosteal bone replaced the inner devitalized cortical bone. In places the combined thickness of old cortical and new periosteal and endosteal bone was 3 times that of the cortex of the control femur.

This layer of new compact bone was definitely and regularly delimited from the original cortex and its origin from the periosteum was substantiated by the prolific production in the presence of abundant nutrition and by the paucity of new osseous tissue where the periosteal blood supply was diminished. Interference with nutrient blood vessels outside the cortex did not produce sufficient nutritional disturbance of the bone to require or stimulate periosteal osteo-

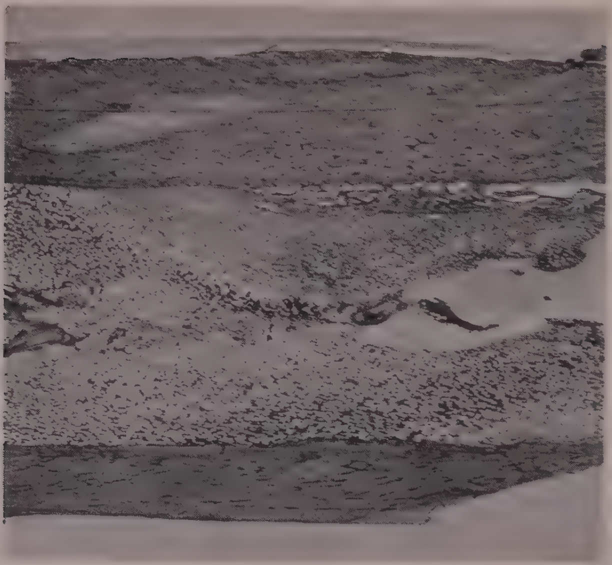

FIG. 1.
Marked periosteal and endosteal osteogenesis in the shaft of a rabbit's femur 49 days after infarction by charcoal emboli (H. and E. x11).

genesis. Mechanical injuries, such as fractures and partial resections of bone or periosteum, which are commonly used in studying bone regeneration, interrupt the continuity or interfere with the nutrition of the structures so that it is difficult to determine the relative importance for healing of the various tissues. Infarction by emboli prevents collateral circulation in the medullary canal and produces necrosis of those tissues that do not receive blood from the periosteum without interrupting the continuity of cortex or periosteum. Necrosis in the inner layers of the cortex and formation of new compact bone in the periosteum after infarction of the medullary tissues alone, and the presence of necrosis of the entire bone with the absence of new osseous tissue after infarction of both the medulla and periosteum, emphasize the necessity of periosteum for viability of the outer portion of the cortex as well as for osteogenesis outside this compact layer.

7510 P

Relative Value of Various Forms of Vitamin D Milk in Prevention of Infantile Rickets.

DONALD J. BARNES. (Introduced by T. F. Zucker.)

From the Children's Hospital of Michigan, Detroit, Michigan.

Antirachitic agents obtained from different sources but containing identical numbers of rat units appear to have different values for various species when used in the prevention or treatment of rickets. Steenbock *et al.*[1] have shown the difference that exists in the reaction of chickens to irradiated ergosterol, irradiated yeast and natural fish liver oils. Our own experience[2] with infants, which has since been amply confirmed, sufficiently demonstrated this difference when the same daily rat unit dose of specially assayed cod liver oil and irradiated ergosterol were used. Hess[3] has shown satisfactory results with small prophylactic doses, *i. e.*, 40 to 50 rat units per day, when taken as irradiated milk.

Accepted standards for the proper dosage of cod liver oil, irradiated ergosterol and irradiated milk are still based largely upon clinical opinion rather than controlled observational material. The statement of Hess[4] is that the relative equivalent rat unit dosage is: of cod liver oil, 240 rat units; viosterol, 600 rat units; irradiated milk, 40 rat units. His conclusions seem to have been arrived at without careful comparative studies having been made. It was with the idea of approaching the minimal prophylactic dosage of cod liver oil vitamin D that this study, giving each infant daily 50 Steenbock units of vitamin D as the Zucker[5] cod liver oil concentrate (emulsified in milk), was made. It also affords an opportunity to compare a carefully measured intake of cod liver oil vitamin D with vitamin D from other sources. This makes no provision obviously for the occasional refractory case that is best safeguarded by a more liberal intake of vitamin D.

The study conducted during the winter of 1932-33 shows the following:

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[1] Steenbock, H., Kletzien, S. W. F., and Halpin, J. G., *J. Biol. Chem.*, 1932, **97**, 249.

[2] Barnes, D. J., Brady, M. D., and James, E. M., *Am. J. Dis. Child.*, 1930, **39**, 45.

[3] Hess, A. F., and Lewis, J. M., *J. Am. Med. Assn.*, 1932, **99**, 647.

[4] Hess, A. F., and Lewis, J. M., *J. Am. Med. Assn.*, 1933, **101**, 181.

[5] Zucker, T. F., Pappenheimer, A. M., and Barnett, M., PROC. SOC. EXP. BIOL. AND MED., 1922, **19**, 167; Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1922 **20**, 136; Zucker, T. F., *Am. J. Pub. Health*, 1933, **23**, 10.

Thirty-two normal infants were completely protected against rickets from November and December to April on 50 units of vitamin D given as concentrate in milk.

Six infants entering the study showed slight X-ray signs of rickets. These healed gradually and definitely through the period of study, and in no case did the X-ray signs become worse.

Of the 38 infants in the study 19 were under 6 months of age and 19 were between 6 months and one year. Of the 19 who were under 6 months of age 11 were under 4 months of age. Weight gains were satisfactory, being on the average about a pound a month. No antirachitic substance or treatment had been previously given.

A control group of 25 infants, who had received no antirachitic treatment during the winter, were selected at random as they came to the clinic. They were comparable in age and size to the groups studied and were X-rayed in April. Fourteen of these, or 56%, showed active rickets, the remainder being normal.

In this group of 38 infants, 50 rat units of vitamin D as cod liver oil concentrate, emulsified in milk, afforded protection against rickets. No difference was found in the older and younger age groups. According to these results the ratio of 40 to 240 for the vitamin D of irradiated milk and the vitamin D of cod liver oil concentrate, emulsified in milk, does not hold. The units seem, for infants, to compare about equally as far as their relative efficacy is concerned. Considering the relatively small number of infants, the error of biological assay, and the difficulties of quantitative clinical evaluation it would not be safe to say that one differs from the other. However, we feel that a larger number of patients must be studied before so low a daily vitamin D intake should be generally recommended. If satisfactory clinical comparisons are to be made between various antirachitic substances it will be necessary to set up and follow a standardized procedure for the various studies made.

7511 C

On the Protein Nature of Prolactin and of Follicle-Stimulating Hormones.

ROBERT W. BATES, OSCAR RIDDLE, AND ERNEST L. LAHR.

From the Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, L. I.

The methods used in the separation and purification of prolactin suggest that this hormone is a protein, but since it has not yet been obtained in a pure state the possibility of its adsorption on inert protein is not thus excluded. It is therefore desirable to learn whether prolactin is affected by tryptic digestion.

That prolactin is rapidly destroyed by trypsin is shown by the data of Table I. Almost complete destruction in 2 hours by a purified trypsin is obtained. The prolactin preparation used in this test was relatively pure and highly potent. Both the control and digest were, at the beginning of the test, at pH 8.0 and were similarly kept at 37° C. At pH 8.0 we have repeatedly found that prolactin will withstand boiling for 1 hour with only slight or moderate loss of potency. No earlier observations on the effect of digestive enzymes on prolactin have been reported.

TABLE I.

Destruction of prolactin (preparation No. 237) by digestion with trypsin (2 hours at 37° C.). Tests made on crop-glands of immature ring doves (material injected in 4 equal daily doses and weights taken 96 hours after first injection).

Preparations	Test animal	Mg. prolactin	Crop-gland weights, mg.		Potency in bird units
			Untreated controls	Treated	
Prolactin, no trypsin	A	16	300-400	2,400	400
	B	16	,, ,,	2,410	
Prolactin + trypsin (digest)	C	16	,, ,,	410	2
	D	16	,, ,,	380	
	E	16	,, ,,	430	
	F	16	,, ,,	610	

The rapid destruction of follicle-stimulating hormone (F.S.H.), obtained from the anterior pituitaries of beef by methods previously described,[1] is shown by the data of Table II. The testes of both birds given F.S.H., heated at pH 8.0 but not subjected to trypsin, are indicated as having increased by something more than 500%. The testes of all of the 4 birds receiving the trypsin digest (birds

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[1] Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

TABLE II.

Destruction of follicle-stimulating hormone (preparation No. 243) by digestion with trypsin (4 hours at 37° C.). Tests made on the testes of immature (2.1-2.9 months) ring doves (material injected in 4 equal daily doses and weights taken 96 hours after first injection).

Preparations	Test animal	Mg. F.S.H.	Weights of testes, mg. Average control for age	Weights of testes, mg. Treated
F. S. H., no trypsin	G	4	8.7	49.2
	H	4	6.5	34.0
F. S. H. + trypsin (digest)	I	8	6.3	6.6
	J	8	6.3	8.8
	K	4	6.5	6.8
	L	4	7.8	10.7

I and J got double dosage) showed only or relatively insignificant gains in weight. Since size increase in testes of the immature dove represents a specific response to the F.S.H. (here the weight is not increased by a Prolan B factor) this result has definite significance. Under these conditions still other relatively pure preparations of F.S.H. have been shown to withstand heating to 37° for 2 and for 4 hours with little loss of potency. A quite different test for F.S.H. potency has been used in another study: that test likewise shows that trypsin rapidly destroys the F.S.H. derived from both pituitary and pregnant urine.

It has recently become evident that one substance (Prolan A) which has long been recognized in pregnant urine is the equivalent of the F.S.H. of the pituitary. Reiss and Haurowitz[2, 3] have previously shown that F.S.H. from both sources is digested by trypsin (completely in 3 or 20 hours) and not by polypeptidases. The mouse ovary and uterus served as test objects for potency in their studies. Our tests made on the true F.S.H therefore confirm earlier work.

Summary. Prolactin is a protein-like substance which at pH 8.0 is almost completely destroyed by trypsin in 2 hours at 37°C. In confirmation of previous work, though using the bird testis instead of the rodent ovary to test potency, it is found that the follicle-stimulating hormone obtained from the anterior pituitaries of cattle is very rapidly destroyed by trypsin under the above-named conditions.

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[2] Reiss, M., and Haurowitz, F., *Z. Ges. Exp. Med.*, 1929, **68**, 371.

[3] Reiss, M., Schäffner, A., and Haurowitz, F., *Endokrin.*, 1931, **8**, 22.

7512 C

Some Physical Properties of the Nuclear Membrane.

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From the Department of Biology, St. Louis University.

During the last 50 years several investigators have described processes which bring into evidence such physical properties of nuclear membranes as resistance to deformation, extensibility, elasticity, etc. (Albrecht,[1] Mottier,[2] Kite,[3] Chambers,[4] Nemec[5]). Our observations made on resting nuclei of the embryonic region of the onion root tip, centrifuged at 30,000 g., (35,000 r.p.m., radius: 22 mm.) are a contribution to the stock of facts already accumulated by these authors.

The root tips, cut off at a length of 18 mm. were fastened by their cut end in a brass tube 15 mm. long and slightly wider than the roots; their free ends were in the centrifugal direction. The tubes were fixed firmly to the rotor of a Sharples electric super-centrifuge. The rotor contained some Knop's plant medium in order to keep the roots steadily immersed. The centrifugation lasted from 10 minutes to 2 hours. The roots were then fixed in Flemming's fluid; and stained either with Heidenhain's iron haematoxylin or Flemming's triple stain.

After a short centrifugation, the nuclear content is concentrated at the bottom of the nuclei and a clearer space appears at the top. Then the whole nuclei are thrown to the bottom of the cells. (Fig. 1 A.) So far these results are the same as those obtained by previous investigators working with a much lower centrifugal force.

After a longer centrifugation the nucleus flattens against the floor of the cell, taking a more or less hemispheric shape, eventually forming an angle of less than 90° between the horizontal diameter of the hemisphere and the vertical portion of the nuclear membrane (Fig. 1B). The diametric line is perfectly straight and shows no evidence of any folding or shrinking. At the top of these hemispheric nuclei one can almost always observe an elevated portion of the membrane which encloses a transparent region and reminds one of a blister. The elevated portion takes often the shape of a

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[1] Albrecht, E., *S. Ges. Morph. Phys., München,* 1898, **14**, 133.

[2] Mottier, *Annals of Bot.,* 1899, **13**, 325.

[3] Kite, G., *Biol. Bull.,* 1913, **25**, 1.

[4] Chambers, R., *Biol. Bull.,* 1918, **34**, 121.

[5] Nemec, B., *Protoplasma,* 1929, **7**, 423.

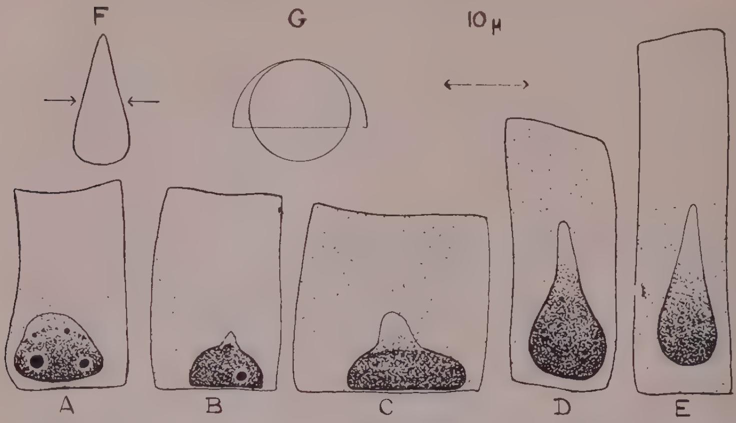

FIG. 1.

A-E, camera lucida drawings of deformed nuclei, centrifuged: *A*, 10 min., *B* and *C*, 30 min.; *D* and *E*, 2 hours; *F* and *G*, diagrams illustrating the discussion. The scale applies only to the five drawings.

conical beak (Fig. 1C and D) pointing towards the center of the cell. The length of the beak is often more than the diameter of the nucleus. The membrane is thin in the elevated portion; it becomes abruptly thick on the spherical part of the nucleus and is still thicker on the diametric line. In some longer cell (Fig. 1 E) the centrifuged nucleus does not reach the bottom of the cell even in two hours; the shape of the nucleus is that of a bottle gourd with a long neck.

The transformation of a sphere into a hemisphere of the same volume requires that the membrane expand its area by 19% (Fig. 1G). The formation of the neck in bottle gourd shaped nuclei increased the length of the peripheric line, as seen in a plane section, by 68% in the specimens with the longest neck; the base of comparison being the average peripheric length of uncentrifuged spherical nuclei. Since the distension appears to be localized in a small area, the coefficient of ductility is apparently very high. The absence of wrinkles or of a reversed curvature on the diametric side of the hemispheric nuclei, where the surface from spherical became plane, might indicate a possible local reduction of area by contraction or by fusion of the wrinkles; but since the total area of the nucleus is increased, no sure conclusion concerning contractility can be reached. We do not think that the differences in the thickness of the membrane can be used to show ductility or contractility; they are probably due to differences of thickness in the optical section.

The fact that no rupture has ever been observed, either in the hemispheric form on the weak points constituted by the right angles or in the bottle gourd forms at the points where the pulling action of the heavy portion of the nucleus is exerted with more intensity (Fig. 1F, arrows) shows a certain mechanical resistance.

In general the physical properties of the nuclear membranes described here are of the type found in interfaces between 2 immiscible fluids of different specific gravity and viscosity.

7513 C

Absence of Antiviral Substance in Normal Adults for the Virus of the St. Louis Encephalitis Epidemic.*

MAURICE BRODIE.†

From the Department of Bacteriology, New York University, and Bellevue Hospital Medical School and Bureau of Health Laboratories, New York City.

Webster and Fite[1, 2, 3] have reported, that on the basis of serological tests, the virus isolated from the St. Louis encephalitis epidemic is not related to that of louping-ill, vesicular stomatitis, equine encephalomyelitis, acute anterior poliomyelitis, Japanese encephalitis (Type B), herpes, or chronic cases of von Economo's disease. The present work confirms and extends some of these findings.

To determine further whether or not there exists any relationship between the virus of the St. Louis epidemic and herpes virus, since there is a possible relationship between the latter virus and that of epidemic encephalitis (reviewed by report of Matheson Commission[4]) both of these viruses were studied. Since neither rabbit, guinea pigs, nor rats could be infected with the encephalitis virus and since animals actively immunized against the latter were found not immune to herpes virus, it was evident that, as far as our tests went, there was no apparent relationship between the 2 viruses.

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* This research was aided by grants received from the New York Foundation and Rockefeller Foundation.

† Fellow in Medicine, National Research Council.

[1] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

[2] Webster, L. T., and Fite, G. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 344.

[3] Webster, L. T., and Fite, G. L., *Science*, 1934, **79**, 254.

[4] Epidemic Encephalitis—Report of a Survey by the Matheson Commission, New York, Columbia University Press, 1929, 1932.

In the following experiments, to test a serum for antibodies, the serum was mixed with equal quantities of virus suspension of various concentrations. The mixtures were incubated at 37°C. for 1 hour during which time they were agitated frequently. Then 0.03 cc. was injected intracerebrally into mice. Simultaneous virus titrations were carried out. A serum which failed to neutralize 1-10 minimal infective doses of virus was said to be devoid of neutralizing substances.

A series of 16 cases of chronic encephalitis, of 1 to 14 years duration and all showing Parkinsonian syndrome, were tested for antibody content. One patient was a Montreal resident, the others attended the Bellevue Hospital Neurological Clinic. Nine of the group had a history of a definite acute onset. None of these serums had neutralizing substances.

As cases in the St. Louis outbreak resembled the Australian X disease in many respects, and since, as was pointed out previously,[5] Breinl isolated the virus of poliomyelitis from 2 cases of the Australian X disease, poliomyelitis antiserums were tested for encephalitis antibodies. Two human convalescent serums, a normal adult serum, a monkey convalescent serum, and the serums of both an actively immunized horse and monkey were tested. All of these serums had a high antiviral action against the virus of poliomyelitis, but none neutralized the encephalitis virus. Moreover, *Macacus rhesus* monkeys, which are highly susceptible to poliomyelitis virus, proved fairly resistant to encephalitis virus.

Since from a Japanese epidemic of encephalitis,[6, 7] a virus related to or perhaps identical with rabies virus, was isolated by Kobayashi,[6] the serums of 3 animals immunized against rabies virus and having demonstrable antibodies against the virus were tested. None of the serums neutralized the encephalitis virus.

The serums of normal individuals, 9 from St. Louis, and 68 from New York were tested. None of these people had any history of exposure to the virus. Five of the serums were from infants and the remainder from adults, whose ages ranged from 20 to 50. All of these serums failed to neutralize the encephalitis virus. Four of these serums were obtained from workers in this laboratory, 2 of whom had worked continuously with the virus. Tests were first carried out when the virus was introduced into the laboratory and retests were done some 3½ months later. The results of all the tests were negative.

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[5] Brodie, M., *Am. J. Dis. Child.*, in press.

[6] Kobayashi, A., *Japan M. World*, 1925, **5**, 145.

[7] Cowdry, E. V., *J. Exp. Med.*, 1927, **45**, 799.

That von Economo's disease and the St. Louis outbreak of encephalitis are due to different etiological agents is quite evident from these experiments and those of Webster and Fite.[2] This is further borne out in the difference in the seasonal and age incidence of the two diseases. Moreover, up to the present, no Parkinsonian-like sequellae have been reported from the 1933 outbreak in St. Louis, nor from similar cases reported in Paris, Ill., in the previous year.

Antiviral substances have been reported in the serums of recovered individuals and in those of physicians and nurses who have been in close contact with cases.[8] The fact that the serums of 9 individuals from St. Louis and 68 from New York, all without a history of contact, had no demonstrable neutralizing substance, suggest that the encephalitis antibody is specific and the result of exposure to the virus. It appears, therefore, that contact with the virus can result in either the disease or immunity, the latter being due, perhaps to reaction with the virus or a subclinical attack of the disease. In the present work there was no evidence to show that exposure to the mouse passage virus gave immunity, although the intracutaneous inoculation of virus into mice gave a high degree of immunity.[‡]

7514 C

Experiments with Virus of the St. Louis Epidemic of Encephalitis.[*]

MAURICE BRODIE.[†]

From the Department of Bacteriology, New York University, and Bellevue Hospital Medical School and Bureau of Health Laboratories, New York City.

Workers have reported the successful transmission of a virus isolated from cases of the St. Louis epidemic of encephalitis to monkeys[1] and to monkeys and mice.[2, 3] Through Dr. Holden of the

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[8] Barr, David L., Meeting Amer. Coll. Physicians, Chicago, 1934.

[‡] I wish to thank Dr. Theodore C. Hempelmann who furnished the serums obtained from St. Louis residents and Dr. Josephine B. Neal, for her helpful interest in the work.

[*] This research was aided by grants received from the New York Foundation and Rockefeller Foundation.

[†] Fellow in Medicine, National Research Council.

[1] Muckenfuss, R. S., *Am. J. Pub. Health*, 1933, **23**, 1148.

[2] Muckenfuss, R. S., Armstrong, C., and McCordick, H. A., *Pub. Health Rep.*, 1933, **48**, 1341.

[3] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

College of Physicians and Surgeons was obtained some of the virus used by Dr. Armstrong which had undergone 13 serial passages in white mice. The intracerebral infective dose of the virus varied between 0.03 cc. $\times 10^{-3}$ to 0.03 cc. $\times 10^{-6}$ during the course of this work.

The present work deals with experimental passage of the virus, its preservation, and its resistance to germicides. White mice showed a fairly uniform susceptibility to the virus. After 3 to 5 days' incubation, the animals developed a hyperirritability, ataxia, convulsions and paralysis. Mice receiving attenuated virus or borderline infective doses developed very mild attacks, with tremors lasting only a day or two. Of 4 such animals tested, one showed immunity against subsequent infection.

Attempts to pass the virus to guinea pigs, rabbits, rats, kittens and ferrets were negative. Intracerebral and intraperitoneal inoculations in 5- to 6-weeks-old rats and 24-hour to 6-months-old guinea pigs were done and in many instances were redone at intervals.

One monkey, given 1 cc. intracerebrally (motor cortex) and 2 cc. intraperitoneally of a 10% suspension of the 26th passage in mice, developed a rise in temperature after 4 days, followed by a general slowness and awkwardness, tremors and ataxia. When sacrificed 3 days later, these symptoms were still manifest. Since others had found the maintenance of the virus strain through monkeys difficult, it was decided to use for serial passage those parts of the monkey's central nervous system that contained the most virus.

To determine this, the following areas were tested for virus by either intracerebral or combined intracerebral and intraperitoneal inoculations in mice:—different areas of the cerebral cortex, *e. g.*, Rolandic, frontal, parietal and temporal regions; cerebellum including both cortex and ganglia; basal ganglia, hippocampus, mid-brain, brain stem, cervical, lumbar and dorsal cord. Only the frontal region, mid-brain and lower cord had demonstrable virus, in small amounts. A concentrated suspension of frontal lobes produced merely tremors and then recovery, or the typical attack only after a prolonged incubation period; while suspensions of the mid-brain and lower cord, both of which produced an attack in mice in the usual incubation period, failed to infect at higher dilution than 1 in 5.

A monkey which received a thick suspension of mid-brain intracerebrally and intraperitoneally, developed no symptoms after this injection nor after a reinjection 9 days later of a similar amount of a suspension of mid-brain and lower cord. Subsequent test showed no antiviral substances in this animal's serum, although it had received 2 intraperitoneal injections of virus suspension, thereby corroborating the low virus content of the inoculums. The serums of relatively insusceptible animals, such as the monkey, and of animals refractory to the virus, were tested for neutralizing substances. The tests were carried out as given in the previous paper. The serums of 3 monkeys, 2 rats, 2 kittens, 6 rabbits, and 5 guinea pigs showed no antiviral action for they failed to neutralize from 1-10 infective doses of virus.

To compare various methods of preserving the virus, and to maintain an even distribution for comparative work, the following methods were tried, *viz.*, emulsified brain, kept both at ice-box temperature (3-6°C.) and frozen; brain tissue ground well in an equal amount of glycerine (Schering-Kahlbaum) and kept at ice box temperature or frozen; and whole brain in glycerine at ice box temperature. By the latter method, suspensions were made using several pieces of a few brains.

Mouse brain suspension frozen with or without glycerine retained its infectivity for at least 3 months, although there was a loss of approximately 90% of the potency the first 2 weeks. None of the 3 methods used at ice box temperature were satisfactory, for the virus was non-infective in a month or less, and at the end of 2 weeks approximately 99% of the infectivity was lost.

The resistance of the virus contained in a suspension of infective brain tissue, to both formalin and phenol, was tested; the former at 20-23°C. and at 5-7°C., the latter at 5-7°. Equal parts of a 10% brain suspension and double the required amount of germicide were mixed. The mixture was shaken frequently. Mice were injected intracerebrally, and intracerebrally and intraperitoneally with the germicidally treated virus suspension.

The virus was quite resistant to phenol for it was still infective after being in contact with 1% phenol for 25 days and 0.5% phenol for 50 days, at ice box temperature.

The virus was much more susceptible to the action of formalin. This germicide in a concentration of 0.1% rendered the virus non-infective between 12-18 hours at room temperature and from 7-9 days at 5-7°. Likewise, 0.2% formalin inactivated the virus between 9 and 12 hours at 20° and 35 days at 5°.

These experiments confirm and extend the findings of Webster[3] that the mouse is the only susceptible animal among the common small laboratory animals. Moreover, the mouse is decidedly more susceptible to the virus than is the monkey. The monkey showed a diffuse distribution of virus in the frontal region, mid-brain and lower cord. No attempt was made to correlate these findings with the histological picture in the monkey central nervous system because an attempt to transmit the virus in serial passage through the monkey failed. However, this finding is in keeping with the diffuse distribution of lesions found in the human.[4] The insusceptibility of other laboratory animals to the virus may be explained by a tissue insusceptibility rather than humoral antiviral action.

7515

Production of a Hyperimmune Antipoliomyelitic Horse Serum.*

M. SCHAEFFER.† (Introduced by William H. Park.)

From the Laboratories of the Department of Bacteriology, New York University, and Department of Health, New York City.

It is generally accepted that immune serum is of little or no therapeutic benefit in poliomyelitis, as well as in other filterable virus diseases. However, it may be of value prophylactically, but this has not as yet been fully demonstrated. Successful passive immunization against some virus diseases leads one to suppose that such protection may possibly be afforded against poliomyelitis when an antiserum of sufficient potency is produced. Numerous attempts[1-4] with varied degrees of success, have been made to prepare an effective antiviral serum in large animals. The production of a potent antipoliomyelitic horse serum was first accomplished in 1929 in these laboratories[5] and at about the same time in England.[6] More recently, Howitt[7] and Schultz and Gebhardt[8] have reported exceptional response toward immunization treatment with poliomyelitic virus in the sheep, goat and horse.

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[4] McCordock, H. A., *Am. J. Pub. Health*, 1933, **23**, 1152.

* This work was begun by the author in the fall of 1931 with the late William B. Brebner in the laboratories of Dr. E. V. Cowdry at Washington University, St. Louis. Supported by grants from Eli Lilly and Company and the Milbank Fund for the Study of Infantile Paralysis.

† The author wishes to express his gratitude to Dr. Wm. H. Park, Dr. E. V. Cowdry, and Mr. W. A. Jamieson for constant advice and encouragement in this work and wishes to thank Dr. Maurice Brodie for confirming the unusually high titres using a different neutralization test method.

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **55**, 662.

[2] Dixon, S. G., and Rucker, J. B., *J. Infect. Dis.*, 1918, **23**, 543.

[3] Pettit, A., *Compt. rend. Soc. biol.*, 1918, **81**, 1087; *Bull. gen. de Therap.*, 1925, **176**, 389.

[4] Neustaedter, M., and Banzhaf, E. J., *J. Am. Med. Assn.*, 1917, **68**, 1531.

[5] Weyer, E. R., Park, W. H., and Banzhaf, E. J., *Am. J. Path.*, 1929, **5**, 517; *J. Exp. Med.*, 1931, **53**, 553.

[6] Fairbrother, R. W., *Brit. J. Exp. Path.*, 1930, **11**, 43.

These investigators used suspensions of infected monkey brain or cord, as antigen. This was inoculated into the animals frequently and over long periods. A resultant serum, upon clinical trial in the New York poliomyelitis epidemic of 1931, proved to contain a toxic factor (probably a nerve tissue antibody). Toxicity tests conducted by the author upon several such serums showed that 2 of them were highly toxic for monkeys in 10 cc. doses with fatal results in some cases within 8 hours following intraperitoneal injection. The toxic effect of one of these serums was also observed by Kramer.[9]

The obvious need of a protein-free virus extract led to the successful attempt to purify virus emulsions by adsorption and elution.[10] Immunization of horses was then begun with such eluates. Three horses were selected and their serums tested for the presence of natural viricidal substance before treatment was begun. All these serums were negative. The horses were inoculated at weekly intervals by the intrasplenic route, this being chosen on the basis of Brebner's observations on the relation of the spleen to immunity to poliomyelitis.[11] Starting with 20 cc. amounts given weekly, the doses were gradually increased until 200 cc. doses were reached and continued to finish out a 6 month period of intrasplenic treatment. Intramuscular and subcutaneous inoculations given at about weekly intervals were then instituted and continued for another period of 9 months. Bleedings were taken at intervals and *in vitro* neutralization tests, to detect the quantity of antiviral substance, were made. All of these tests were carried out according to the method described by Shaughnessy.[12]

The results may be briefly summarized as follows:

Horse No. 1246—Serum of this horse showed a neutralization titre of 1:5 (serum:virus) after first month of treatment. Failed to show increase in titre after 4 months of further treatment. Discarded and replaced by Horse No. 1204.

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[7] Howitt, B. F., *J. Infect. Dis.*, 1932, **50**, 26.

[8] Schultz, E. W., and Gebhardt, L. P., *J. Immun.*, 1934, **26**, 93.

[9] Kramer, S. D., personal communication.

[10] Schaeffer, M., and Brebner, W. B., *Arch. Path.*, 1933, **15**, 221.

[11] Brebner, W. B., *Am. J. Path.*, 1931, **7**, 546; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 351; and numerous unpublished observations made in conjunction with author.

[12] Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 463.

Horse No. 1204—Serum showed an increasing rise in neutralization titre, reaching maximum of 1:40 after 6 months. Subsequent subcutaneous and intramuscular injections failed to increase potency of its serum.

Horse No. 1247—Serum reached maximum neutralization titre of 1:60 after 6 months of treatment. Subsequent intramuscular and subcutaneous inoculations raised the potency of its serum to a titre of 1:100. The concentrated pseudoglobulin of this serum has a neutralizing power of 1:1200.‡

The serum and concentrate is not toxic for monkeys even when given in doses as large as 90 cc.

Preliminary experiments on passive immunization, with the serum concentrate, indicate that it may be of value, since monkeys have been protected against intracerebral doses of highly infective virus for several days.

Two new sets of horses are now being immunized by different methods with the hope of obtaining even more potent products.

7516 C

Modification of Vagus Inhibition of the Heart by Quinidine.

M. H. NATHANSON.

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Quinidine acts directly upon the myocardium and is a powerful cardiac depressant. The drug depresses auriculo-ventricular conduction and retards the rate of the sinus node in dogs.[1] Auricular irritability is definitely diminished in the terrapin's heart.[2] However, when applied to clinical cases of hyperactivity of the sinus node as in sinus tachycardia, quinidine does not consistently exhibit its depressor effect. The rate of the sinus rhythm is frequently increased rather than retarded.

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‡ This serum is 3 to 4 times as potent, and the concentrate about 40 times as potent as human convalescent serum. The concentrate is at least 10 times as potent as the previous one prepared in this laboratory.[5]

[1] Lewis, T., Drury, A. N., Iliescu, C. C., and Wedd, A. M., *Heart*, 1921, **9**, 55.

[2] Hirschfelder, A. D., and Cervenka, C., *J. Pharm. and Exp. Therap.*, 1925, **26**, 19.

Lewis and his associates[1] have demonstrated in dogs that in addition to its direct cardiac action, quinidine also modifies the response of the heart to vagus stimulation. The prolongation of auriculo-ventricular conduction time on vagus stimulation was markedly lessened under quinidine. In some instances vagus stimulation actually shortened the conduction period. The reaction of the sinus node to vagus stimulation was only slightly affected. In the cat, however, Dale[3] showed that the vagus effect on the sinus node was not only eliminated by quinidine, but a reversal of vagus action could be obtained with an acceleration of the sinus rate. In man it is possible to study the effect of vagus stimulation on the sinus node by application of the carotid sinus reflex. In some subjects an intense reflex stimulation of the vagus nerve is obtained[4] with temporary inactivity of the sinus node and cardiac standstill. This reaction can be abolished by atropine. Six subjects were selected in whom a prolonged cardiac standstill could be consistently induced by pressure on the right carotid sinus. These subjects were repeatedly tested to determine the consistency of the response. In each instance a uniform reaction was obtained under repeated application of the reflex. Following a control electrocardiogram recording the effect of right carotid sinus pressure, quinidine sulphate was administered orally for a period of 4 or 5 days in increasing doses. On the final morning of the experiment 1.8 to 2.2 gm. was given in divided doses. One hour after the last dose, an electrocardiogram was made, again recording the effect of carotid sinus pressure. In each case the prolonged standstill (intense vagus effect) was replaced by a slight slowing of the sinus rate (mild vagus effect). Figures 1 and 2.

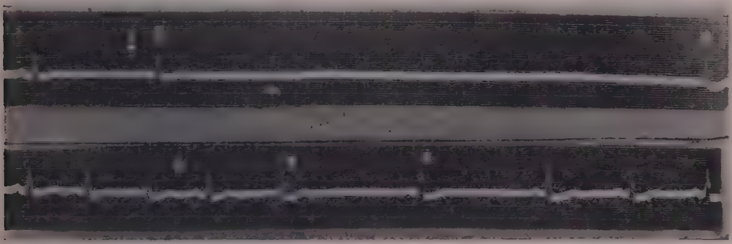

FIG. 1.

Subject H. B. Upper strip shows cardiac standstill (R — R = 9.1 seconds) after pressure on right carotid sinus (arrow). The lower strip after administration of quinidine sulphate 2 gm., maximum R — R = 2.2 seconds.

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[3] Dale, H. H., *Heart*, 1921, **9**, 87.

[4] Nathanson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1037.

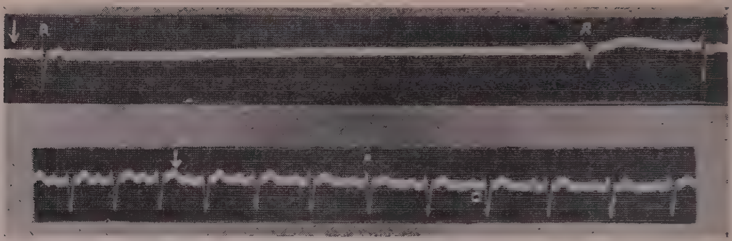

Fig. 2.

Subject J. Q. Upper strip shows cardiac standstill (R — R = 11 seconds) after pressure on right carotid sinus (arrow). Lower strip after administration of quinidine sulphate 2.2 gm. Carotid sinus pressure produces slight slowing of heart rate from 80 to 58 per minute.

These observations indicate that the action of the vagus nerve on the sinus node in the human heart is definitely modified by quinidine. This effect must influence the general reaction of the heart to the drug and may explain the variable results which follow its administration in clinical cases.

Peiping Section.

Peiping Union Medical College, May 16, 1934.

7517 P

Studies on Radiation Leucopenia. I. Effects of Pentnucleotide in Rabbits.

H. T. KIMM AND J. W. SPIES. (Introduced by C. M. Van Allen.)

From the Department of Surgery, Peiping Union Medical College.

Recently, nucleic acid salts have been widely used in the treatment of agranulocytosis with encouraging results. It seems, therefore, that this form of treatment might also be applicable to leucopenia produced by radiation.

Normal, female rabbits of from 3 to 4 months old were used. The leucopenia was induced by roentgen radiation. A single exposure (650 r units corresponding to 100% E.S.D.) of 46½ minutes duration was given over the dorsal aspect of the body excluding the head. The factors were 130 K.V., 4 ma., 6 mm. Al. filtration, 30 cm. skin-target distance. Immediately after the termination of the radiation, 2.5 cc. (0.175 gm.) of pentnucleotide* was injected intravenously and then twice daily for 10 consecutive days. Daily R.B.C. and W.B.C. counts, Hb. determinations (Sahli), and differential W.B.C. counts (Wright's stain) were made with blood taken from the ear vein, at the same hour of the day, both before radiation and for an average period of 4 weeks after radiation. The control series included 8 rabbits; of which 3 received radiation without pentnucleotide, 3 received pentnucleotide without radiation, and 2 were given 2.5 cc. of a watery solution of 0.3% cresol (containing no pentnucleotide) twice daily for 10 days. The weight, temperature, and the general appearance of the animals were noted three times a week.

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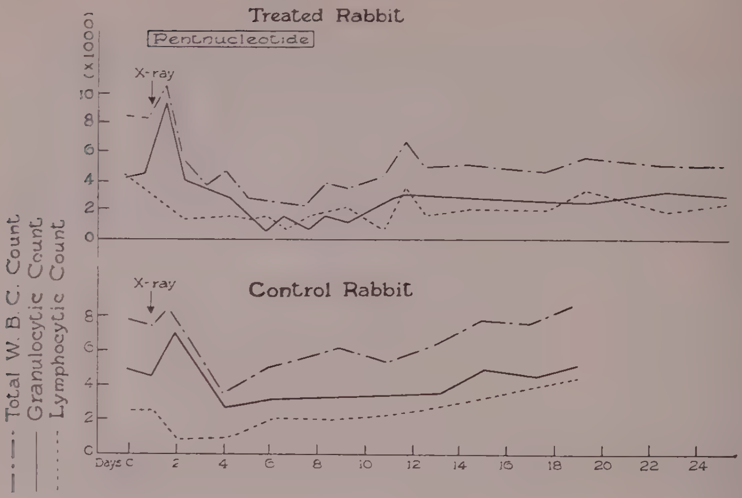
*A watery solution of the sodium salts of pentose nucleotides preserved with 0.3% cresol (Smith, Kline & French Laboratories, Philadelphia).



In the animals that received pentnucleotide alone, there was marked increase in the total number of granulocytes and lymphocytes, which appeared on the sixth to tenth day and lasted throughout the period of observation. The rabbits that received cresol solution alone showed no appreciable change in the W.B.C. count. Those which had radiation alone suffered a definite reduction in the total number of W.B.C. occurring between the 3rd and 5th days following the exposure, after which there was a gradual rise, with return to normal between the fifteenth and seventeenth days. A typical case is represented in the lower chart of the accompanying figure. The rabbits which received radiation and pentnucleotide, displayed essentially the same type of reaction to the radiation as did those of the preceding group. See upper chart of the figure. The general appearance, weight, temperature, R.B.C. count, and Hb. of all the rabbits remained unaffected throughout the period of observation.

It seems that practically no effect can be attributed to the pentnucleotide. This may be due to a depression of the bone marrow by the roentgen rays, independent of the metabolism of nucleic acid.

7518 P

Osteogenic Power of Tibial Periosteum.

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From the Division of Orthopedic Surgery, Department of Surgery, Peiping Union Medical College.

In previous experiments[1] it was shown that a great difference exists in the osteogenic power of the periosteum of the rib and tibia of the same animal. Periosteum from only the mid-portions of these bones was tested. In the present experiments, specimens of periosteum of various portions of the tibia were compared with each other in this respect.

Rabbits, 6 to 12 months old, and adult dogs were used, in 3 groups. In each animal, equal strips of bone-free periosteum were aseptically removed from different parts of the bone's antero-medial aspect and laid upon the fascia of the corresponding tibialis anticus muscle. After 5½ to 10 weeks, the specimens were removed and examined for the extent of osteogenesis.

Group 1, 4 rabbits. The specimens were from the upper and lower thirds of the shaft of the tibia. New bone formed in both, but it was about 5 times more abundant in the specimen from the upper third. In each case, the end of the graft originally nearer the epiphysis had more bone than the other.

Group 2, one rabbit and 2 dogs. The specimens were from the upper, middle, and lower thirds of the bone. Those from the middle third developed no bone; and the others behaved as in group 1.

Group 3, 2 dogs and 2 rabbits. The grafts were from the upper third and from the junction of the middle and lower thirds of the shaft of tibia. The positions of the transplantations were reversed (in order to control the factor of possible differences in vascularity of different parts of the tibialis anticus muscle fascia). The transplants from the upper third of the shaft showed solid sheets of bone, while those from the junction of the middle and lower thirds of the shaft showed only a very slight amount of osteogenesis—slightly less than that formed in the grafts taken from the lower one-third of the shaft as in groups 1 and 2.

These experiments show that free grafts of periosteum from the upper third of the shaft of the tibia have a very marked capacity for forming new bone, that those from the lower third are the next most active, and that those from the middle third are the least active. Apparently, the nearer the source of the graft is to the epiphysis, upper or lower, the greater is the osteogenic power. The differences as to the site of implantation of the graft seemed to be unimportant in this respect.

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[1] Fang, H. C., and Miltner, L. J., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 386.

Clinical observations on the rapidity of the repair of fractures (in man) in these different parts of the tibia correspond closely with the osteogenic power as shown in these experiments.

7519 P

Narrowing of Artery from Contraction of Experimentally Produced, Fibrous Capsule.

C. C. CHANG AND C. M. VAN ALLEN.

From the Department of Surgery, Peiping Union Medical College.

Previously the contractibility of capsules of young sterile fibrous tissue surrounding pulmonary lobes and intestinal segments, organs offering little resistance to the constriction, was studied;[1, 2, 3] whereas the present work concerns the contractibility of such tissue about arterial segments, where far greater resistance occurs.

Fourteen arteries, femoral and carotid, in 6 dogs, were handled. Initial treatment in each case: Under aseptic precautions, a short length of the vessel was exposed by incision and loosely wrapped once around with light, close-woven, collodion-impregnated, silk cloth or with thin rubber dam. This material was 3 cm. long and just wide enough loosely to encircle the vessel; after application, its opposing edges were fastened together with silk sutures. The wound was closed. Seven to 105 days after operation, the vessel was removed *in toto*, injected first with lipiodol at arterial pressure for roentgenographic determination of the size of the lumen, and injected afterward with formalin for fixation and section.

Two of the wounds became infected and the specimens were rejected. In the other cases, the findings were as follows: The envelop, bathed in a little sero-fibrinous exudate, lay loosely about

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[1] Van Allen, C. M., and Wang, T. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 812.

[2] Wolfe, J. J., Wang, T. T., and Van Allen, C. M., *J. Thoracic Surg.*, 1934, **3**, 300.

[3] Van Allen, C. M., and Chao, Y. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1242.

the vessel. All adjacent tissues had developed a layer of fibrous tissue, 1 mm. or less in thickness, next to the foreign material, the layer on the inside of the envelop having developed from the adventitia of the artery and constituting a capsule around the vessel. This layer could be easily stripped from the muscularis. In the younger specimens, it was composed of closely packed, well preserved fibroblasts and, in the older ones of relatively acellular, largely hyalinized fibrous tissue. The muscularis and intima were without cellular change, and the lumen was quite empty. The size of the latter varied greatly, depending on the age of the specimen. Table I gives the calibre of the enveloped part of each vessel relative to that of the non-enveloped, immediately adjacent parts. The 7-, 58-, and 105-day specimens showed no reductions of calibre; but the others were all more or less extensively strictured. Arranged in order of increasing age, the specimens of 10 to 24 days exhibited irregularly progressive reductions, while those of the following 81 days showed irregularly progressive increases in calibre. The silk and rubber seemed to react similarly. Thrombosis was entirely lacking.

TABLE I.

Dog No.	Enveloped Artery	Envelop Material	Period of Envelopment (days)	Cross-sectional Area of Lumen (% of normal)
1	Carotid	Silk	7	100
2	”	”	10	70
3	”	Rubber	14	26
4	Femoral	Silk	14	13
1	”	”	14	25
1	”	”	21	13
5	”	”	21	4
4	”	”	24	4
1	Carotid	”	28	62
2	”	Rubber	58	100
6	Femoral	”	73	28
5	”	”	105	100

The narrowing of the vessels seemed due to a concentric contraction of the fibrous capsules surrounding them. Probably the high intravascular pressure, in the presence of hyaline degeneration of the capsule, was responsible for the dilatation; since in the previous experiments with relatively slight resistance to the cicatricial contraction, the narrowing persisted indefinitely. Attention is recalled to the fact that the cicatrix here represented developed under aseptic conditions, for there is reason from other experiments to suppose that the contractibility of such tissue is very different (much less) than that of infected scar.

The constriction of veins by this process is being studied. These vessels, too, may become extensively compressed without injury to the walls and without thrombosis.

7520 P

Narrowing of Intestine from Contraction of Experimentally Produced, Fibrous Capsule.

C. M. VAN ALLEN AND Y. C. CHAO.

From the Department of Surgery, Peiping Union Medical College.

The constrictive force of a capsule of young sterile fibrous tissue surrounding short segments of intestine was studied, as an extension of previous experiments in the contractibility of this tissue.[1, 2]

Twelve different segments of ileum, 3 in each of 4 dogs, were treated. Operation: The abdomen was opened under aseptic precautions, a loop of terminal ileum was delivered, and a side-to-side anastomosis was made between the limbs of the loop, so as to sidetrack about 40 cm. of the gut. Then the mesentery of the sidetracked portion was incised about 3 cm., parallel to and about .5 cm. from the gut, at 3 different sites about 10 cm. apart and no nearer to the anastomosis than 5 cm. No mesenteric vessels were injured. At one site, the gut with its attached bit of mesentery was wrapped once about with a strip of thin rubber dam 2.5 cm. wide and just long enough to reach loosely around. The opposed edges were sutured together. At another site, light, close-woven silk cloth was used in the same manner; and at the third, this kind of cloth, impregnated with collodion (to render it impervious to fibroblasts), was similarly used. The whole loop was wrapped in omentum, returned to the abdomen, and the abdomen was closed. Five to 20 days after operation, the dogs were killed and the loops were removed. The bowel at each enveloped site was injected, first, with water under a gradient of pressure, to determine the patency; second, with barium suspension under 20 cm. H_2O pressure, to portray by X-ray the outlines of the lumen; and, third, with formalin, for fixation and section.

The reactions to the different kinds of envelop were essentially the same: All tissues in contact with the foreign material developed

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[1] Van Allen, C. M., and Wang, T. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 812.

[2] Wolfe, J. J., Wang, T. T., and Van Allen, C. M., *J. Thoracic Surg.*, 1934, **3**, 300.

a heavy fibrous coating. That part of the coat against the inner aspect of the envelop came from, and completely surrounded, the intestinal segment. A considerable amount of slightly turbid, serofibrinous fluid bathed the envelop within these walls. The envelop was loose, much looser than at first because the gut was greatly narrowed. At 5 days (with injection), the cross-sectional area of the lumen was about 6% of normal and, from the 8th day on, the lumen was fully collapsed. At 8 days, water could trickle easily through; but at 10 days, it had to have at least 8 to 12 cm. H_2O pressure to get through at all, and at 20 days, it failed entirely to pass, even with pressures as high as 60 cm. H_2O. Cross-sections always showed the walls of the enveloped segments to be much compressed and the mucous and muscular layers to be thickened. The mucosa was closely and deeply folded. The muscularis was easily separable from the capsule; and neither mucosa nor muscularis presented evidence of inflammation, necrosis, or other cellular change. The capsule was sharply demarcated and was composed of young fibroblasts, loosely arranged in the earlier specimens and compactly laid in the later ones.

During the first few days, the intestinal narrowing was probably due to the pressure of the encysted surrounding fluid, perhaps assisted by local muscular spasm; but thereafter it was certainly maintained and probably also advanced considerably by the fibrous capsule.

7521 P

Determination of Oxidation-Reduction Potential with Double Electrodes.

TUNG-TOU CHEN. (Introduced by Hsien Wu).

From the Department of Biochemistry, Peiping Union Medical College.

According to the electrode equation,

$$E = E'_0 - \frac{RT}{nF} \ln \frac{[S_r]}{[S_0]}$$

the oxidation-reduction potential, E'_0, of a given oxidation-reduction system at a given pH can be calculated from the potential observed, E, when a blank noble metal electrode is placed in a mixture in which the ratio of the concentration of the total reductant, $[S_r]$, to that of the total oxidant, $[S_o]$, is known. For the benzoquinone-hydroquinone system, the equation becomes

$$E = E'_o - \frac{RT}{2F} \ln \frac{[\text{hydroquinone}]}{[\text{benzoquinone}]}$$

Theoretically the potential is independent of the total concentration of the two components of the system and independent of the size of the electrode.

The mechanism through which the electrode responds to the composition of the mixture is not clear, but in a homogeneous solution the number of the reductant particles acting upon any unit area of the electrode must bear to the number of the oxidant particles acting, the same ratio as that in the solution. Slight difference in the uniformity of the electrode surface should make no difference in the electrode behavior. Assuming the surfaces of the electrode on both sides to be identical, we should obtain the same potential, if we could separate the 2 species of particles and place only pure species on each side of the electrode. Similarly if we could split one electrode into 2 identical halves and immerse them separately in 2 solutions, one containing the pure reductant and the other, the pure oxidant, the potential observed should not differ from that observed when the original electrode is placed in the solution made by mixing the 2 pure solutions.

In the present experiment, this hypothesis was tested using the benzoquinone-hydroquinone system. Instead of splitting one electrode into 2 identical halves, 2 electrodes as nearly identical as possible were prepared. One of them was placed in a solution of benzoquinone of known concentration while the other was placed in a solution of hydroquinone of the same or different concentration. Both solutions were prepared with the same buffer mixture. The 2 electrodes were connected to a common potentiometer lead outside the solution. The results as summarized in Table I are in agreement with the hypothesis. In the table the theoretical potential was calculated from the equation,

TABLE I.

Relation of oxidation-reduction potential to pH of the benzoquinone-hydroquinone system. Temperature: 23° C.

pH	E'_o observed	E'_o theoretical	Difference
1.08	.639	.637	+.002
2.02	.583	.581	+.002
3.01	.523	.524	—.001
4.00	.463	.466	—.003
4.98	.409	.408	+.001

$$E'_0 = E_0 + \frac{RT}{F} \ln (H^+)$$

which becomes, at 23°C.,

$$E'_0 = 0.7007 + 0.05872 \log (H^+)$$

Aside from its theoretical interest, this new arrangement is open to several practical possibilities. Of these 2 may be mentioned. First, in the determination of the oxidation-reduction potential of many quinone-hydroquinone systems, the formation of quinhydrone-like complexes often introduces difficulties in mathematical analysis of the results. With the present arrangement there is no danger of the formation of such complexes. Second, when the 2 components of an oxidation-reduction system to be studied are in separate vessels, the pH values of these two solutions do not have to be the same. Variation of the pH of only one solution at a time may facilitate the study of the dissociation constants of either component.

7522 P

Effect of Carbon Arc Radiation on Bone Healing.

H. MORROW SWEENEY. (Introduced by Henry Laurens.)

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

Ultraviolet radiation and the addition of irradiated materials to the ration are powerful aids to the absorption and utilization of calcium and phosphorus. That such radiation and vitamin D (irradiated ergosterol) induce beneficial effects in the healing of bone fractures is supported by some[1] and denied by others.[2]

This report deals with the results of a study of 38 experimental fractures of the fibula in 25 dogs, and of 80 fractures in 80 albino rats. An open osteotomy technique similar to that of Lindsay and Howes[3] was used. All the rats were of the same size and age and with like fractures. They were grouped for comparison according to the type and amount of carbon arc radiation received during the healing periods. The fractures in the dogs were paired for comparison only when 2 were proved to be very similar or identical by means of roentgenograms: In the same dog serially (12 cases) and in 2 dogs of similar body build and size (7 cases). Measured amounts of carbon arc radiation were given to the dogs during the healing period of one of each pair of fractures, while the other served as a control in 15 of the 19 pairs (38 fractures).

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[1] a. Knoflach, I. G., *Wien. klin. Woch.*, 1928, **4**, 739.

b. Cuthbertson, D. P., *Biochem. J.*, 1930, **24**, 1244.

[2] a. Coulter, J. S., and Smith, E. M., *Radiology*, 1931, **16**, 737.

b. Swart, H. A., *J. Bone and Joint Surg.*, 1930, **12**, 360.

[3] Lindsay, M. K., and Howes, E. L., *J. Bone and Joint Surg.*, 1931, **13**, 491.

Roentgenography was the criterion used for following the healing process. In the rats the percentage healed per group after a certain length of time, was ascertained, while in the dogs the total length of the healing process in days was found. During the total healing period allowed the rats (about 10 weeks) 73.9% of the animals irradiated with "A" carbons (having received 550 gm. cal. per sq. cm. with an average distribution of 3.6% ultraviolet, 32.2% luminous, and 64.2% infrared), 26.9% of those irradiated with "C" carbons (having received 290 gm. cal. per sq. cm. with an average distribution of 5.0% ultraviolet, 11.5 luminous, and 83.5 infrared), and 41.3% of the controls healed. Omitting 2 very abnormal healing periods in the dog work, the average healing time was 55.7 days, or 3.7 days shorter when the animals were irradiated, than when they were not (59.4 days). Both "A" and "C" carbons (see average distribution above) were used on the dogs. The daily amounts given varied from 5 to 28 gm. cal. per sq. cm. with a mean of 18. The total amount varied with the length of the healing period for they were irradiated until healed.

It made little or no difference in the healing time whether the irradiated or control fracture was the first followed, thus the rapidity of healing was not a function of the duration of the experiment. There was no demonstrable correlation between serum calcium and inorganic phosphorus and length of healing time.

Why the healing process of the rats irradiated with "C" carbons was actually retarded is of considerable interest. Although the percentage of ultraviolet emitted by the "C" carbons is greater than for the "A", the total amount in this work was never as much. The "C" carbon has been shown to emit a larger percentage of the short ultraviolet, erythema-producing wave lengths which lead to more marked superficial vasodilatation and possibly increased volume flow of blood. Jones and Roberts[4] believe that an increased flow of blood is detrimental to a bone healing process.

Thus it may be said that in our hands the doses of carbon arc radiation given were of no outstanding importance in accelerating the healing of fractures in dogs and the effect varied with the type of radiation in the rat work.

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[4] Jones, R. W., and Roberts, R. E., *Brit. J. Surg.*, 1933-1934, **21**, 461.

7523

Further Studies on Metabolism of Sulfur Compounds in Cystinuria.*

ERWIN BRAND AND GEORGE F. CAHILL.

From the Department of Chemistry, New York State Psychiatric Institute and Hospital and from the Squier Urological Clinic of the Presbyterian Hospital, New York.

Some recent experiments[1, 2] on sulfur metabolism have been confirmed on another case of cystinuria (16-year-old female from a cystinuric family). *Cystine* (6.4 gm.), administered orally was almost completely (98%) oxidized to inorganic sulfate and did not increase urinary cystine excretion. *Cysteine* (3.5 gm.), on the other hand, was largely excreted as extra cystine (45%) and only a part of it was oxidized (38% of the extra sulfur). *Methionine* (dl, 6 gm.) was also largely excreted as extra cystine (40%) and partly (35%) as undetermined neutral sulfur,† while only a small amount (25% of the extra sulfur) was oxidized.

A preliminary experiment with 2.5 gm. of homocystine[3] indicates that in cystinuria this amino acid is oxidized (92% of the extra sulfur) and does not give rise to extra cystine in the urine. The implications of this finding will be discussed upon completion of confirmatory experiments now in progress on another patient.

The cystine in the urine of this patient, as determined by our modification of the Sullivan method, increased with the ageing of the specimen in agreement with earlier observations.[4]

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*Aided by Grant No. 302 from the Committee on Scientific Research of the American Medical Association.

[1] Brand, E., Cahill, G. F., and Harris, M. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 348.

[2] Brand, E., *Bull. N. Y. Acad. Med.*, 1934, **10**, 289.

† The analyses indicate the excretion of organic sulfur neither in SS nor SH form. It is possible that some unchanged methionine was excreted since the racemic amino acid was fed.

[3] Butz, L. W., and du Vigneaud, V., *J. Biol. Chem.*, 1932, **99**, 135.

[4] Brand, E., Harris, M. M., and Biloon, S., *J. Biol. Chem.*, 1930, **86**, 315.

7524 C

Effect of Caffeine on Basal Metabolism.

NATHAN A. WOMACK AND WARREN H. COLE. (Introduced by E. A. Graham.)

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In a recent report we called attention to the fact that when certain of the methylated purines were fed or injected into dogs morphological evidence of increased activity of the thyroid gland was obtained.[1,2,3] Toxic and frequently fatal doses of these substances were often required to produce such changes. These studies have been extended with especial reference to the calorigenic action of one of these purines, namely, caffeine citrate.

It has been realized for some time that caffeine possesses the ability to increase the oxygen consumption in the organism.[4] The mechanism has been vague although the adrenal glands, the skeletal muscle and the central nervous system have all been suggested as playing some part in this process. The present report has to do with the relation of the thyroid gland to this phenomenon.

Young guinea pigs weighing between 150 and 450 gm. were used. The basal metabolic rate was determined by a method previously described by us in which a closed circuit is used and direct oxygen consumption measured. A normal rate of oxygen consumption was established for each animal before the experiment was begun. These animals were placed on a basal diet of lettuce, oats and alfalfa. Basal metabolism determinations were made on alternate days for 2 weeks before the normal rate of oxygen consumption was considered established. Unless the animal showed 3 successive readings of the same rate the basal rate was not considered established. Each animal was fed 75 mg. of caffeine citrate by mouth per kilo of body weight. Apparently the method of administering the caffeine was of little importance. Determinations were made one hour later. A progressive rise in the basal metabolic rate was noticed which reached a peak at about the end of 2 weeks. At that time the increase was generally from 40%-75%. There was then a gradual decline until by the end of the fourth week the increase was more or less constant at a level of about 20%-25% increase. When the basal

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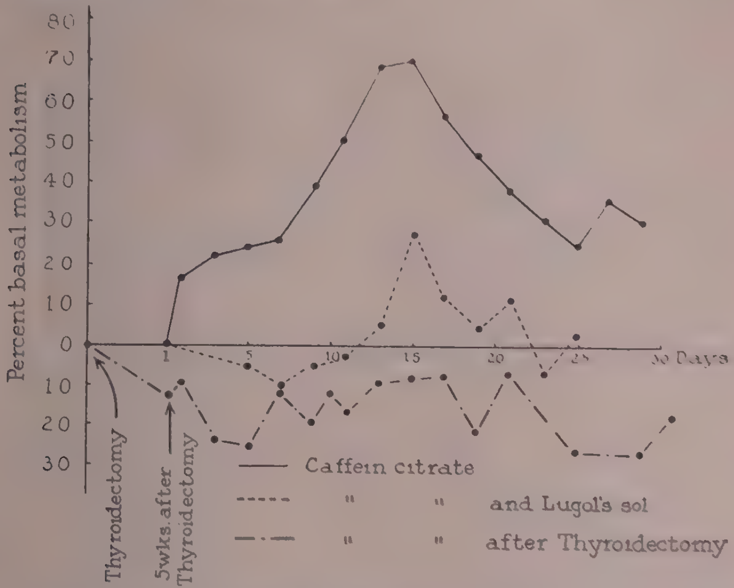
[1] Cole, Womack, and Ellett, *Arch. Surg.*, 1931, **22**, 924.

[2] Edsall and Means, *Arch. Int. Med.*, 1914, **14**, 897.

[3] Higgins and Means, *J. Pharm. and Exp. Ther.*, 1915, **7**, 1.

[4] Means, Aub and DuBois, *Arch. Int. Med.*, 1917, **19**, 832.

metabolism determinations were made 24 hours after caffeine feeding the rise was still seen to be present but not quite so marked. Animals sacrificed at the end of 4 weeks showed little evidence of hyperactivity of the thyroid gland.



Another series of animals was studied under similar conditions with the exception that they were fed one minim of Lugol's solution daily beginning 2 days before the feeding of caffeine citrate and continued throughout the experiment. Very little rise in the oxygen consumption was noticed. When this did occur it was generally most prominent at about the end of the second week and was rarely over 20%-25%. Apparently, therefore, iodine is capable of preventing the calorigenic action of caffeine to a large extent.

A third series of animals in which thyroidectomy had been done was studied. At the end of 5 weeks it was found that the oxygen consumption had fallen from 9%-13% below normal. Caffeine citrate was then fed and basal metabolism determinations made as in series one. A slight rise was seen usually about 10% above the thyroidectomized level but rarely reaching the level of oxygen consumption previous to the thyroidectomy. The animals were sacrificed and serial sections made of the neck. In every case a small amount of accessory thyroid tissue was found although at times this consisted of only several acini. However, none of these animals can be considered totally thyroidectomized.

Conclusions. Caffeine citrate increases the basal metabolic rate of guinea pigs. This increase is present 24 hours after feeding caffeine though not quite so marked. Lugol's solution or thyroidectomy tends to prevent this calorigenic action.

7525 C

Effect of Supersonic Waves on Bacteria.

ALBERT C. H. YEN AND SZU-CHIH LIU. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology and the Department of Biochemistry, Peiping Union Medical College.

Destruction of living organisms by supersonic waves has recently been observed.[1] The mechanism of the destruction is, however, unknown. The present communication extends the observation to a number of pathogenic and nonpathogenic bacteria and the results seem to indicate that the destruction is due to the dissolution of the bacterial cells.

The oscillating circuit was similar to that described by Wu and Liu,[2] and the quartz was adjusted to vibrate at the rate of 1.5×10^6 times per second. Bacterial suspensions to be exposed were placed in a thin test tube, 15 mm. in diameter. Inside the test tube was placed a glass cooling coil through which cold water circulated. The temperature of the bacterial suspension was never over 20° C. The possibility of destruction by heat was therefore eliminated.

Saline suspensions of the following 10 strains of bacteria were used: *Bacillus subtilis, Bacillus anthracis, Bacillus proteus X* 19, *Bacillus coli communis, Bacillus typhosus, Bacillus dysenteriae Shiga, Staphylococcus aureus, Micrococcus catarrhalis, Bacillus influenzae,* and *Streptococcus hemolyticus.* The number of surviving bacteria per cc. before and after exposure was determined by counting the colonies in poured plates. In the case of *Bacillus influenzae* streak plating was used. Control experiments with bacterial suspension standing at room temperature without exposure showed no significant change in the number of surviving bacteria. Results are shown in Fig. 1.

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[1] Wood, R. W., and Loomis, A. L., *Phil. Mag.*, 1927, **4**, 417. Harvey, S. N., and Loomis, A. L., *J. Bact.*, 1929, **17**, 373. Beckwith, T. D., and Olson, A. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 362. Williams, O. B., and Gaines, N., *J. Inf. Dis.*, 1930, **47**, 485.

[2] Wu, H., and Liu, S. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 782.

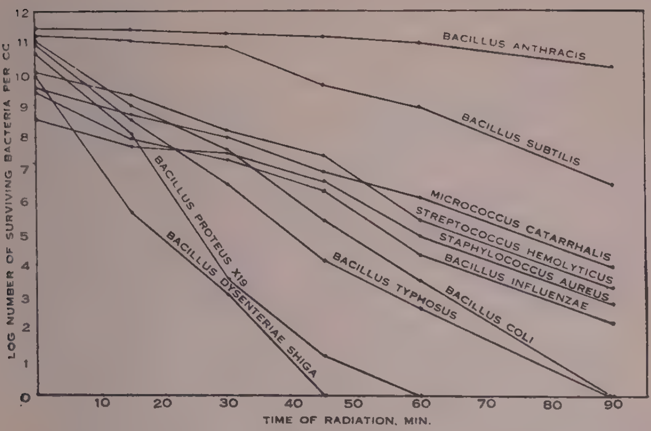

Fig. 1.

Effect of exposure on number of surviving bacteria. Volume of bacterial suspension used = 1 cc.

It will be noted that the organisms studied can be divided into 3 groups according to their susceptibility to the supersonic wave. The first group represented by *Bacillus anthracis,* is practically not affected by a 90-minute exposure. The second group which includes *Micrococcus catarrhalis, Bacillus subtilis, Streptococcus hemolyticus, Bacillus influenzae,* and *Staphylococcus aureus,* is partially destroyed but not completely sterilized by a similar exposure. The third group which includes *Bacillus proteus X* 19, *Bacillus coli communis, Bacillus typhosus* and *Bacillus dysenteriae Shiga,* is completely sterilized within 90 minutes. The organisms of this group are members of the Gram negative intestinal bacilli. The ability to form spores does not seem to explain the high resistance of *Bacillus anthracis* to supersonic waves, because the other spore-bearing organism studied, *Bacillus subtilis,* was definitely destroyed.

In order to throw some light on the mechanism we have compared the opacity, using Pulfrich photometer, with the number of surviving bacteria in a few of the suspensions before and after exposure. Results are shown in Fig. 2.

It will be noted that, in the case of *Staphylococcus aureus,* where little change of the number of surviving bacteria was observed, the opacity did not show appreciable change in the course of a 90-minute exposure. In the case of *Bacillus proteus* and *Bacillus dysenteriae Shiga,* destruction of bacilli was accompanied by a definite decrease of opacity in the first 30 minutes. This suggests that destruction of bacteria by supersonic wave was accompanied by lysis. In other words, destruction is due to the dissolution of the bacterial cells.

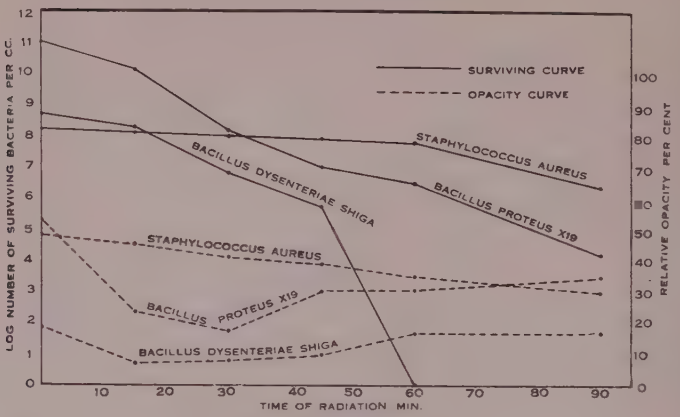

FIG. 2.

Relation between relative opacity and number of surviving bacteria. Volume of bacterial suspension used = 5 cc.

On longer exposure the number of surviving bacteria continued to decrease while the opacity increased to a definite level and then remained practically constant. This increase of opacity is probably due to the coagulation[2] of the cell proteins after lysis.

This study shows that exposure to supersonic waves brings about the killing and dissolution of various bacteria and that on prolonged treatment coagulation of the dissolved bacterial protein may be effected.

7526 C

Variation in Potency of Vaccinia Virus in Tissue Cultures.

W. K. CH'EN. (Introduced by T. J. Kurotchkin.)

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The possibility of employing vaccinia virus in pure culture for prophylactic vaccination against small-pox has been indicated by Rivers,[1] Herzberg[2] and Stevenson and Butter.[3] This leads to investigation of the behavior of such cultures, especially their immunizing and infective properties. Therefore a study was undertaken to determine the effect of repeated passages in chick-embryonic tissue culture on the potency of the vaccinia virus.

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[1] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

[2] Herzberg, K., *Klin. Wochenschr.*, 1932, **11**, 2064.

[3] Stevenson, W. D. H., and Butter, G. G., *Lancet*, 1933, **21**, 228.

The initial virus was prepared by passing the calf-lymph vaccinia virus supplied by the National Epidemic Prevention Bureau, Peiping, through the testicles of a normal rabbit. The bacteria-free vaccinia virus thus obtained was cultivated in the chick embryonic tissue according to the method described by Rivers.[1] Transfers of the virus to fresh chick embryonic tissue were made every 5 days. After each transfer the titre of the virus, *i. e.,* the lowest dilution which still gave the typical skin lesion was determined by intradermal injections of 0.25 cc. of varying saline dilutions of the virus culture into the shaved skin of albino rabbits. The first virus culture gave a titre of 1:1,000 which rose to 1:100,000 on the 3rd and 4th transfers and reached the peak of 1:1,000,000 on the 5th transfer after which it declined as subsequent transfers were made so that by the 25th transfer the titre had dropped to 1:1,000, equaling that of the initial virus, and by 30th transfer it had fallen to 1:100. From this point on the titre remained at a level between 1:50 to 1:100 when carried up to the 40th transfer. As no further drop in titre was observed, it was concluded that the virus culture had reached a stage of biological stability at the low titre of 1:50 to 1:100.

Since it is well known that the calf-lymph vaccinia virus of low titre can be increased by interposing a rabbit passage, it appeared interesting to see whether passage of this low titre vaccinia virus through a rabbit testicular tissue culture could raise the titre of the virus. The virus cultures after 30 and 40 transfers in chick embryonic tissue were inoculated separately into minced young rabbit testicular tissue cultures in Tyrode's solution. After the first passage, the titre immediately rose to 1:1,000 and by the third passage in the testicular medium it became 1:100,000. On reinoculating these virus cultures to chick embryonic tissue, the titre of the virus remained at the high level of 1:1,000, 1:10,000, 1:100,000 respectively after the 1st, 2nd, and 3rd transfers. Thus, it was clearly shown that the vaccinia virus of low titre after repeated passage in chick embryonic tissue culture could be made to regain its high titre by an interposing passage through a rabbit testicular tissue culture.

To determine further the immunizing power of the virus cultures after repeated passages in chick embryo, the following experiment was performed. Several rabbits were divided into 3 groups and each group of animals was immunized by intradermal vaccinations. The first group was given intradermal injection with a virulent virus culture (titre 1:1,000,000) obtained after the 5th passage in chick embryonic tissue medium, the second group with a virulent virus culture (titre 1:10,000) grown in rabbit testicular tissue medium and the third, with a virus of low virulence (titre 1:100) obtained after the 35th passage, in chick embryonic tissue culture. The test for immunity was performed one month later by intracutaneous injections of varying dilutions of a virulent vaccinia culture. The subsequent observation of all these animals clearly demonstrated that no appreciable differences in the state of immunity could be detected among the 3 groups. It was found that each group of animals showed immunity reaction with the dose equal to 1:100 dilution or lower of the virulent culture, while an entirely negative response was observed with a 1:1,000 dilution of the culture. Control test with normal rabbits showed that typical skin lesions were produced when a 1:10,000 dilution of the same culture was inoculated.

The result suggests that in rabbits the production of vaccinia immunity does not necessarily require the use of a highly virulent culture. Adequate immunity in rabbits can easily be created with a virus of low virulence. Therefore in human practice of prophylactic inoculation against small-pox, the use of a vaccinia virus of low virulence may present several advantages, one of which concerns the existence of post-vaccinal encephalitis. Without going into the details of this particular problem, one may, on the basis of the theoretical conception, assume that such a pathological condition can more successfully be brought about when a virus of high potency is used. To substantiate this assumption we have performed an experiment in which the invasive power of a vaccinia culture of high and low potency has been determined. Several rabbits were injected either intratesticularly or into the brain, using a desired quantity of the respective culture and it has been found that 2 rabbits injected into the brain and 2 rabbits injected into the testicles with a highly virulent culture succumbed to the infection; out of 4 rabbits similarly inoculated with a slightly less virulent culture only one animal survived; while, on the other hand, all 6 rabbits which received the intracranial or intratesticular inoculations of a low virulence culture survived. It would appear definite that the attenuation of the vaccinia virus cultivated for 30–40 generations in the chick embryonic tissue culture is associated with the entire loss of its invasive potency, while as shown above it still retains its protective power.

There may be objections to the practical employment of the vaccinia virus of low virulence considering the question of the "take".

This important question can be solved only by the actual test on human beings. It seems to us, however, that this objection is partially obviated by the observation that for many years the attenuated calf vaccine has been found satisfactory as regards its "take."

7527 C

A Simple Method for Aseptic Grinding of Bacteria.

K. C. MAK. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

For the mechanical disintegration of bacteria Macfadyen and Rowland[1] employed a highly-complicated machine in which the organisms are pulverized in a frozen condition between a rapidly rotating metal pestle and a stationary metal receptacle immersed in liquid air. The bacteria are rendered very brittle by this intense chilling and are rapidly disintegrated by the grinding process. The amount of bacteria dealt with is not large, varying from 0.5 to 1 gm. of the washed organism. Barnard and Hewlett[2] introduced a ball mill capable of grinding bacteria in a moist pasty condition. Thomson[3] devised a vaccine churn that is capable of smashing bacteria in emulsion form with some success. Recently, Krueger[4] described a type of ball mill for disintegrating bacteria and tissues under aseptic conditions.

The grinding machines above described are generally very costly to construct, and are not all uniformly successful. For grinding small quantities of bacteria it appears that an inexpensive and equally efficient apparatus may be used with advantage. A simple method in which bacteria may be disintegrated in a moist condition is here described.

The grinding chamber consists of a large-size pyrex test tube measuring approximately 20 cm. long and 3 cm. wide. Into this are placed a number of rustless steel balls, 8 mm. in diameter, sufficient to fill one-third of the test tube. The tube is tightly closed, plugged by means of a rubber cork and inserted into an outer metal tube 25 cm. long and 3.2 cm. wide. The test tube is held firmly in position inside the metal tube by means of a rubber cork as shown in Fig. 1. The test tube will now move with the metal tube when the latter is rotated. The outer rubber cork so effectually seals up the metal tube that in case of breakage (which practically never happens) the bacterial contents cannot escape.

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[1] Macfadyen, A., and Rowland, S., *Centralbl. f. Bakt.*, 1903, **34**, 765.

[2] Barnard, J. E., and Hewlett, R. T., *Proc. Roy. Soc. B.*, 1912, **84**, 57.

[3] Thomson, D., *Annals Pickett-Thomson Res. Lab.*, 1924, **1**, 275.

[4] Krueger, A. P., *J. Inf. Dis.*, 1933, **53**, 195.

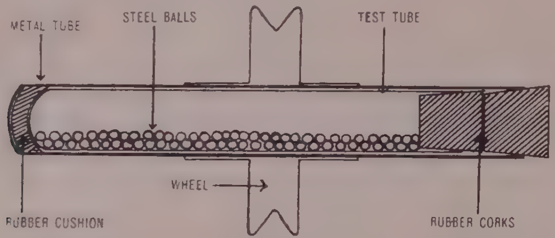

FIG. 1.
Cross section of the grinding apparatus.

Into the middle of the metal tube a wooden pulley wheel, 12 cm. in diameter, is fixed by means of a screw. The grinder is supported by means of a pair of metal rings on a retort stand so that it is capable of rotating in a horizontal axis (Fig. 2).

Power is derived from an electric motor, developing ⅛ H.P. such as is found in most laboratories. It should preferably be fitted with a speed regulator, but failing this, the grooved wheel of the motor may be replaced by one with a very narrow diameter, altering the gear ratio to such an extent that the grinder will describe approximately 350 rotations a minute.

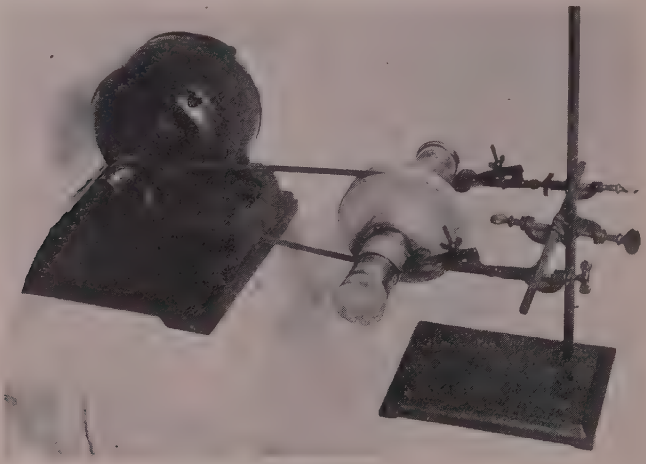

FIG. 2.
Showing the grinding apparatus in operation.

The retort stand is tightly clamped to the table to minimize vibrations, and the moving parts are well-oiled to reduce friction.

Fig. 2 represents the apparatus properly fitted up for grinding (clamp not shown in photo). Bacteria from 15 or more Kolle flasks are washed repeatedly with saline in a centrifuge tube and then emulsified in 15 cc. of saline. The emulsion is pipetted with aseptic technic into the sterile grinding chamber which is then held in position inside the metal tube. Grinding is continued at 350 rotations a minute for from 20 to 70 hours. The period of grinding may be shortened or lengthened at will, depending on the thickness of the emulsion and the resistance of the organisms to grinding. When the organisms have been efficiently ground, as judged by smear examination, a sterile extract may be made by high speed centrifuging to remove intact bacteria, or by filtration through a sterile candle. It may be necessary to dilute the extract a few times to facilitate filtration, as the volume of extract is small.

To obviate the trouble of drying the bacteria we have attempted to grind them in a moist condition. Disintegration in emulsion form, is, according to Thomson, much cleaner and more convenient than dry disintegration, and our experience supports this view. The organisms are introduced into the grinder in the form of a thick emulsion so as just to fill the interspaces between the steel balls. Larger volumes retard the grinding as the bacteria at the surface of the emulsion are not subjected to the tumbling effect of the balls.

However, the apparatus is also capable of grinding bacteria in a dry powdery condition. This has the advantage of being able to deal with a larger quantity of bacteria.

Artificial cooling is not necessary with this apparatus. There is but little heat formation during the grinding process, and at no time has the temperature risen above 37° C.

Staphylococcus aureus, B. diphtheriae, B. tuberculosis, B. proteus, and yeast cells have been disintegrated by this method. Yeast cells are very easily ruptured, and 5 hours' grinding removes about 95% of the intact cells from sight. A microscopic examination at this time shows but a few intact cells per field and an abundance of gram-negative debris derived from the ruptured cells. Staphylococci are much more resistant and a much longer grinding is necessary in order to bring about a satisfactory result. Grinding for 40 hours removes about 85% of the intact cocci as judged by microscopic examination. A 20 hour grinding is sufficient to cause 50% of the staphylococcal proteins to go into colloidal solution as found by the Van Slyke method.

The apparatus fulfils all the ordinary requirements of a miniature bacteria grinder. It grinds in an aseptic manner, requires no artificial cooling, is entirely devoid of danger, and is simple to use. It has the advantage of being easily constructed as most of the working parts are to be obtained in a well-equipped laboratory, and it is thus inexpensive.

7528 C

Cultivation of Leishmania Donovani in Media of Embryonic Chick Tissues.

ALBERT C. H. YEN AND HUEI-LAN CHUNG. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology and the Department of Medicine, Peiping Union Medical College, Peiping.

For the cultivation of *Leishmania donovani* a number of media generally containing blood have been advocated. In such media only flagellate forms are found. It is of interest to record the growth of Leishmania flagellates in media of various embryonic chick tissues which have not hitherto been used for the purpose.

The chick embryos employed were fertilized hen's eggs incubated at 37°C. for a varying period of 4 to 20 days. The embryonic chick brain, heart, liver and intestines were removed separately and chipped into fine pieces with sterile scissors in different dishes. They were then suspended in sterile Tyrode's solution having a pH 8.0 in approximate concentration of 0.5 gm. of tissue to 10 cc. of fluid. In some experiments the whole chick embryo from 4 to 7 days old minced finely and suspended in Tyrode's solution was used. With a sterile capillary pipette one drop (about 0.15 cc.) of the embryonic tissue suspension was placed in the center of sterile cover-glass. To this was added a similar drop of Tyrode's solution containing *Leishmania donovani* from the spleen of an infected hamster. A sterile hollow glass slide measuring 7.5 cm. long, 2.5 cm. wide, and 0.7 cm. thick, with a central pit or well, 1.8 cm. in diameter and 0.4 cm. deep, was smeared with sterile vaseline around the mouth of its well. The hollow glass slide was then inverted over the cover-glass in such a way that the inoculated media fluid on the cover-glass faced the center of the well of the hollow glass. The whole slide together with the cover-glass was then turned over by quick motion so that the hanging drop did not spread to the periphery but remained in the center of the under-surface of the cover-glass. Pressure was gently applied to the latter to ensure airtight sealing of the hanging drop inside the hollow glass. Many such hanging drop preparations were made and incubated at 37°C. and at 20°C. in separate lots. The advantage of such hanging drop cultures is obvious. It permits direct microscopic examinatiion at frequent intervals without contamination.

Growths of flagellates were easily demonstrable in the chick brain, heart and whole embryonic tissue preparations from the fifth day on when the cultures were kept at 20°C. Similar preparations kept at 37°C. consistently yielded negative results. In number and size the flagellates reached their maximum at the end of 2 weeks, beyond which the number began to diminish. Generally after 5 weeks it became difficult to demonstrate the presence of the well preserved flagellates due apparently to the exhaustion of the nutriment in the hanging drop or possibly to the accumulation of toxic metabolic products.

Multiplication of the Leishmania flagellates in these embryonic chick tissue media were shown by the successful subcultures from the first positive hanging drop to a fresh hanging drop preparation. Such subcultures at 20°C. generally showed increase of flagellates in large numbers, which became obvious from the 7th day on but there was no growth at 37°C. Attempts to subculture in hanging drops the flagellates from N.N.N. media were also successful only at 20°C.

Of interest is the fact that the media containing either embryonic chick liver or intestine alone repeatedly showed no growth of flagellates. When the tissue suspension was made up of equal parts of chick liver and chick brain, the flagellates also failed to grow.

7529 C

Flagellation of Leishmania Donovani in Blood from Normal and Infected Hamsters.

HUEI-LAN CHUNG. (Introduced by F. R. Dieuaide.)

From the Parasitic Diseases Laboratory, Department of Medicine, Peiping Union Medical College.

Hitherto the cultivation of *Leishmania donovani* has been on various artificially prepared media usually with rabbit's blood as one of the ingredients. It is of some interest to record the flagellation of this parasite in blood from normal and kala-azar hamsters.

A minute piece of spleen from a hamster heavily infected with kala-azar was ground up in a mortar with 1.2 cc. of heart blood from the same animal. From this 4 hanging drop preparations were made with aseptic technique and sealed airtight with sterile vaseline. Two of these preparations were kept at a temperature between 20° C. and 22° C. These showed a large number of Leishmania flagellates when examined on the 6th day. The remaining 2 preparations kept at 37°C. showed no flagellates on repeated examination at different intervals up to the end of 3 weeks. Similar experiments repeated with normal hamster's blood yielded the same results, namely, the development of flagellates in such hanging drop preparations when kept at room temperature (20°C.-22°C.) but none in the preparations kept at 37° C.

The range of temperature in which flagellation may occur may be quite large, for Christophers, Shortt, and Barraud[1] obtained positive cultures of *Leishmania donovani* in temperatures between 16° C. and 34° C. The fact that *Leishmania donovani* from the spleen of a kala-azar hamster develop into flagellates in the blood from the same animal at room temperature points to the possibility that the parasite may even flagellate in the mammalian host under favorable conditions.

7530 P

Relationship of Peptic Ulceration to Gastric Chemism.

ALTON OCHSNER, MIMS GAGE, AND KIYOSHI HOSOI.

From the Department of Surgery, Tulane University School of Medicine, New Orleans, La.

The relationship of gastric acidity to peptic ulceration has been debated for years. The present investigation was undertaken in an attempt to evaluate the etiologic importance of gastric acidity. A total of 125 dogs was used, but of this number 68 died at various intervals after the operation, leaving 57 animals on which our results are based. Each animal was kept in separate clean quarters with a constant supply of fresh drip water. They were allowed to play on the roof on sunshiny days. All the lesions described were submitted to microscopic study. Gastric analysis was done about 6 times before operation and then once a week postoperatively after a standard test meal of cracker dust. The following series of dogs were used as controls or received thrice daily every other day either 1% HCl feeding or 1/25 to 8/10 mg. histamine injections subcutaneously: I. No operative procedures were done to get the gastric reaction to the HCl feeding or histamine injections. II. Extirpation of (a) greater curvature; (b) lesser curvature. III. Formation of a gastric pouch from (a) greater curvature of Heidenhain type, or (b) lesser curvature (Pavlov type) plus anastomosis of the proximal jejunum to the pouch. IV. Same as series III plus anastomosis of fundus of gall bladder to the pouch.

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[1] Christophers, S. R., Shortt, H. E., and Barraud, P. J., *Ind. Med. Res. Memoirs*, 1924-1927, **1-5**, 55.

Repeated HCl feeding produced multiple acute lesions—petechiae, erosions and shallow ulcers of the stomach and duodenum, which were observed as early as 5 days after starting the feedings. In the longer experiments (2 months), there was associated a mild to marked gastritis and duodenitis with increased mucus production. In general there was a tendency toward temporary achlorhydria with a return to normal values of free HCl after a month or so of experimentation. Histamine injections also gave similar results. Histamine was found to produce multiple acute ulcers but did not stop them from healing. In one animal, which had received injections over a period of 134 days, there were 22 ulcers of various sizes and depths, some showing microscopic evidence of healing. Ulcers were most frequently or most abundantly found along the lesser curvature. The acidity values obtained were usually within the range of variations normal for dogs, although sometimes the acidity values after histamine stimulation were frequently double that of the pre-operative values without histamine stimulation. The incidence of acute ulcers in series I was 27%.

Extirpation of the greater or lesser curvature of the stomach in dogs did not significantly change the acidity values of the gastric contents of the main stomach when compared with the pre-operative values. HCl or histamine injections produced findings similar to those in series I. The incidence of acute ulcer in series II was also 27%.

In series III, subacute or chronic jejunal ulcers were obtained in 100% of the greater curvature pouch experiments and in 71% of the lesser curvature pouch dogs, or an average of 85%. As regards the location of these anastomotic ulcers, 71% of these were in the efferent loop, 14% opposite the gastro-jejunostomy stoma, and 14% marginal. The proximal or afferent loop was always normal in appearance, whereas the distal or efferent loop showed the effects of the irritating acid gastric pouch secretion passing through it. It was edematous, congested, with numerous variously sized petechiae and hemorrhages and even erosions. Histamine injections produced 100% of anastomotic ulcers in this series of either the greater or lesser curvature experiments. Sixty-six per cent of the animals in this series died from perforation peritonitis.

In series IV, bile appeared to be a good neutralizing agent, since it reduced the incidence of post-operative anastomotic ulcers from 100% to 43% in the greater curvature pouch dogs and from 71% to 50% in the lesser curvature pouch animals, giving an average of 39% ulcer incidence for this series. Eighty per cent of these ulcers perforated. As regards the lesions of the stomach or duodenum itself, deviation of the bile toward the pouch in 13 dogs produced hemorrhages and erosions in the main stomach in only one animal and a chronic duodenal ulcer in another, both without histamine, and a small pyloric mucosal hemorrhage in one with histamine stimulation. The experiments of series III and IV indicate that while acidity of the gastric secretion may not be the sole predisposing factor, it is nevertheless a major exciting factor whenever condition favorable for ulceration prevails, such as allowing acid secretions of the gastric pouch to flow into the jejunum which is not accustomed to such acid environment.

TABLE I.
Incidence of Ulcers in Our Experiments.

Group I. Control group, no operative procedures.				
		1% HCl	Histamine	Controls
		8 dogs	7 dogs	
	Ulcers	1	3	—
	Erosions	4	1	
Group II. Extirpation of greater or lesser curvatures.				
		3 dogs	4 dogs	4 dogs
Greater curvature	Ulcers	0	2	2
	Erosions	2	1	0
			1 dog	3 dogs
Lesser curvature	Ulcers	—	0	0
	Erosions	—	0	0
Group III. Gastric pouch plus jejunal anastomosis.				
			3 dogs	3 dogs
Greater curvature	Ulcers		3	3
			3 dogs	4 dogs
Lesser curvature	,,		3	2
Group IV. Gastric pouch plus jejunal anastomosis plus gall bladder.				
			4 dogs	3 dogs
Greater curvature	Ulcers		2	0
			3 dogs	3 dogs
Lesser curvature	,,		0	3

SECRETARY'S ANNUAL REPORT

April 1, 1933 to April 1, 1934

Announcement is made of the formation of the Southern California Section, composed of members in or near Los Angeles.

Authors are reminded that to expedite publication each manuscript should indicate whether it is preliminary or complete. Preliminary manuscripts should be not longer than 2 pages, inclusive of tables and charts. In special instances the Board of Editors may make an exception. This involves delay in publication.

There were 617 manuscripts published during the fiscal year, of which 58 per cent were preliminary and 42 per cent complete.

MEMBERSHIP

Members, March 31, 1933	1135	
Elected during year	106	
Honorary Members	11	
Total	——	1252
Resignations	18	
Deaths	9	
Arrears	7	34
	——	——
Total Membership, March 31, 1934		1218

	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934
Membership	692	779	851	936	986	1026	1062	1138	1146	1218

SUBSCRIPTIONS

Subscriptions, March 31, 1933	417
Subscriptions, March 31, 1934	432
Total No. of Proceedings Distributed 3/31/33	1563
Total No. of Proceedings Distributed 3/31/34	1650

OFFICERS elected April 18, 1934

President: E. L. Opie.
Vice-President: P. E. Smith.
Secretary-Treasurer: A. J. Goldforb.
Council: E. DuBois.
H. W. Smith
W. B. Cannon
F. R. Sabin } Hold over
C. R. Stockard
G. B. Wallace
Past Presidents (by constitution): E. B. Wilson, S. Flexner, F. S. Lee, T. H. Morgan, J. Ewing, W. J. Gies, G. Calkins, G. B. Wallace, J. W. Jobling, S. R. Benedict, P. Rous, D. J. Edwards, A. R. Dochez.
Chairmen of Sections (See Sections).

Nominating Committee (elected April 18, 1934): P. Rous, Chairman, E. Allen, A. J. Carlson, A. R. Dochez, E. A. Doisy, R. Gesell, A. C. Ivy, J. R. Murlin, O. Riddle.

Editorial Committee (appointed by Council): S. Bayne-Jones, A. J. Carlson, M. H. Jacobs, C. A. Kofoid, H. B. Lewis, A. B. Luckhardt, W. deB. MacNider, J. R. Murlin, A. M. Pappenheimer, H. B. Williams, A. J. Goldforb.

Membership Committee (appointed by Sections): E. R. Becker, W. R. Bloor, L. J. Cole, H. Cummins, E. A. Doisy, H. Goldblatt, I. Greenwald, A. C. Ivy, W. P. Larson, E. G. Martin.

Financial Committee: W. J. Gies, W. H. Park, A. J. Goldforb.

PAST OFFICERS

Date	*President*	*Vice-President*	*Secretary*	*Treasurer*
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	W. J. Gies	G. N. Calkins
1905-06	E. B. Wilson	E. K. Dunham	W. J. Gies	G. N. Calkins
1906-07	S. Flexner	E. K. Dunham	W. J. Gies	G. N. Calkins
1907-08	S. Flexner	T. H. Morgan	W. J. Gies	G. N. Calkins
1908-09	F. S. Lee	T. H. Morgan	W. J. Gies	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	G. Lusk
1910-11	T. H. Morgan	W. J. Gies	E. L. Opie	G. Lusk
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	G. Lusk
1912-13	J. Ewing	P. A. Levene	G. B. Wallace	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	C. Norris
1914-15	G. Lusk	W. J. Gies	H. C. Jackson	J. R. Murlin
1915-16	G. Lusk	G. N. Calkins	H. C. Jackson	
1916-17	J. Loeb	W. J. Gies	H. C. Jackson	
1917-18	W. J. Gies	J. Auer	H. C. Jackson	
1918-19	W. J. Gies	J. Auer	H. C. Jackson	
1919-20	G. N. Calkins	G. B. Wallace	H. C. Jackson	
1920-21	G. N. Calkins	G. B. Wallace	H. C. Jackson	
1921-22	G. B. Wallace	J. W. Jobling	H. C. Jackson	
1922-23	G. B. Wallace	J. W. Jobling	H. C. Jackson	
1923-24	H. C. Jackson	J. W. Jobling	V. C. Myers	
1924-25	H. C. Jackson	J. W. Jobling	A. J. Goldforb	
1925-26	J. W. Jobling	S. R. Benedict	A. J. Goldforb	
1926-27	J. W. Jobling	S. R. Benedict	A. J. Goldforb	
1927-28	S. R. Benedict	P. Rous	A. J. Goldforb	
1928-29	S. R. Benedict	P. Rous	A. J. Goldforb	
1929-30	P. Rous	D. Marine	A. J. Goldforb	
1930-31	P. Rous	D. J. Edwards	A. J. Goldforb	
1931-32	D. J. Edwards	A. R. Dochez	A. J. Goldforb	
1932-33	A. R. Dochez	E. L. Opie	A. J. Goldforb	
1933-34	A. R. Dochez	E. L. Opie	A. J. Goldforb	

SECTIONS

Cleveland, Ohio

Chairman: C. S. Beck. Secretary: A. R. Moritz. Members: 37.

Meetings: Western Reserve University, October 13, 1933.
Western Reserve University, November 10, 1933.
Western Reserve University, December 8, 1933.
Western Reserve University, January 12, 1934.

Illinois

Chairman: Lloyd Arnold. Secretary: W. L. Palmer. Members: 103.

Meetings: University of Chicago, October 10, 1933.
University of Illinois College of Medicine, January 16, 1934.
Northwestern University Medical School, April 3, 1934.

Iowa

Chairman: P. C. Jeans. Secretary: W. F. Loehwing. Members: 32.
Meetings: State University of Iowa, February 16, 1934.
State University of Iowa, March 23, 1934.
Iowa State College, April 26, 1934.

Minnesota

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 46.
Meetings: University of Minnesota, October 18, 1933.
University of Minnesota, November 15, 1933.
University of Minnesota, February 21, 1934.
University of Minnesota, April 18, 1934.
University of Minnesota, May 16, 1934.

Missouri

Chairman: D. P. Barr. Secretary: E. A. Doisy. Members: 44.
Meetings: Washington University Medical School, October 11, 1933.
St. Louis University Medical School, November 8, 1933.
Washington University Medical School, January 10, 1934.
St. Louis University Medical School, March 14, 1934.
Washington University Medical School, May 9, 1934.

New York

Chairman: E. L. Opie. Secretary: A. J. Goldforb. Members: 703.
Meetings: New York Academy of Medicine, October 18, 1933.
New York Academy of Medicine, November 15, 1933.
New York Academy of Medicine, December 20, 1933.
New York Academy of Medicine, January 17, 1934.
New York Academy of Medicine, February 21, 1934.
New York Academy of Medicine, March 21, 1934.
New York Academy of Medicine, April 18, 1934.
New York Academy of Medicine, May 16, 1934.

Pacific Coast

Chairman: C. L. A. Schmidt. Secretary: M. L. Tainter. Members: 73.
Meetings: Stanford University, October 14, 1933.
University of California Medical School, December 13, 1933.
Stanford University Medical School, February 14, 1934.
Mount Zion Hospital, San Francisco, April 18, 1934.

Peiping (China)

Chairman: R. K. S. Lim. Secretary: S. H. Liu. Members: 25.
Meetings: Peiping Union Medical College, October 25, 1933.

Southern

Chairman: Charles F. Craig. Secretary: R. H. Turner. Members: 36.
Meetings: Hotel John Marshall, Richmond, Va., November 15, 1933.
Tulane University, February 2, 1934.
Tulane University, May 11, 1934.

Southern California

Chairman: J. F. Kessel. Secretary: T. D. Beckwith. Members: 25.
Meetings: University of California at Los Angeles, May 26, 1934.

Western New York

Chairman: F. P. Knowlton. Secretary: W. S. Root. Members: 52.
Meetings: Clifton Springs Sanitarium, October 21, 1933.
Syracuse University, December 16, 1933.
University of Rochester, February 17, 1934.
University of Buffalo, May 5, 1934.

Wisconsin

Chairman: E. L. Sevringhaus. Secretary: M. R. Irwin. Members: 31.
Meetings: University of Wisconsin, January 31, 1934.
University of Wisconsin, March 13, 1934.
Marquette University Medical School, May 18, 1934.
University of Wisconsin, June 2, 1934.

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: Doctors N. C. Davis, C. H. Eckles, N. B. Foster, F. L. Gates, A. F. Hess, A. B. Macallum.

A. J. GOLDFORB,
Secretary.

TREASURER'S REPORT

April 1, 1933 to April 1, 1934

RECEIPTS

Balance on Hand, April 1, 1933		$ 3,131.75
Income, 1933-34:		
Dues	$ 4,860.96	
Reprints	2,749.87	
Excess Space	1,736.43	
Cuts	552.24	
Subscriptions	2,662.08	
Back Numbers Sold	282.57	
Bank Interest and Miscellaneous	38.07	
Total Annual Income		$12,882.22
Total Cash Available April 1, 1933-April 1, 1934		$16,013.97

DISBURSEMENTS

Publication Cost of Proceedings:		
Printing	$ 5,228.27*	
Reprints	2,484.98†	
Cuts	542.38‡	
		$ 8,255.63
Administrative Expenses:		
Office Supplies, Postage and Telegrams	$ 523.55	
Salaries	2,025.00	
Storage and Insurance	78.47	
Refunds	5.90	
Miscellaneous	38.22	
		$ 2,671.14
Total Annual Disbursements		$10,926.77
Cash Balance April 1, 1934		5,087.20
Total		$16,013.97

SUMMARY

Income (net)		$12,882.22
Disbursements (net)	$10,926.77	
Unpaid bills	848.89	
Surplus for year		$ 1,106.56

*Plus $507.88 not yet paid.
†Plus $283.09 not yet paid.
‡Plus $ 57.92 not yet paid.

$848.89

FUNDS

Endowment Fund

April 1, 1933	$14,016.02	
Interest to April 1, 1934	424.49	
Total		$14,440.51

Invested in N. Y. Title and Mortgage Co.	$ 6,000.00	
Invested in Title Guarantee and Trust Co.	2,500.00	
Invested in Lawyers Mortgage Company	1,500.00	
Invested in R. R. Coop. Bldg. and Loan	984.93	
Invested in Bowery Savings Bank	425.68	
Invested in Industrial Bonds (purchase price)	3,029.90	
(Present value, $3,162.50)		$14,440.51

Special Surplus Fund

April 1, 1933	$ 7,578.08	
Interest to April 1, 1934	305.13	
Total		$ 7,883.21

Invested in Title Guarantee and Trust Co.	$ 2,900.00	
Invested in R. R. Coop. Bldg. and Loan	2,620.74	
Invested in Bowery Savings Bank	229.50	
Invested in Industrial Bonds (purchase price)	2,132.97	
(Present value, $2,145.00)		$ 7,883.21

Life Membership Fund

Invested in R. R. Coop. Bldg. and Loan	$ 75.00

MEMO

Bills payable	$ 848.89
Bills receivable	$ 866.62

Auditors' Report

April 11, 1934

We have examined the Treasurer's report and have verified the statements therein against the books of the Society. We are convinced that the records of financial transactions are in good order and accurately kept.

(Signed) H. D. SENIOR
A. M. PAPPENHEIMER

MEMBERS' LIST (Alphabetical)

Honorary Members

Councilman, William T.	Harvard University
Halliburton, W. D.	London, England
Howell, William H.	Johns Hopkins University
Lombard, Warren P.	University of Michigan
Porter, William	Harvard University
Reichert, E. T.	University of Pennsylvania
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Schaffer, E. S.	Edinburgh, Scotland
Von Muller, Friedrich	Munich, Germany
Wilson, Edmund B.	Columbia University

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Adams, William E.	University of Chicago
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Adler, Herman M.	Berkeley, California
Adolph, E. F.	University of Rochester Medical School
Adolph, W. H.	Peiping, China
Albus, William R.	American Type Culture Collection, Chicago
Alexander, Harry L.	Washington University
Allen, Bennet M.	University of California
Allen, Edgar	Yale University
Allen, Ezra	N. Y. Homeopathic Medical College
Allen, William F.	University of Oregon
Alles, G. A.	Los Angeles, California
Alsberg, Carl L.	Stanford University
Alt, Howard L.	Northwestern University Medical School
Althausen, T. L.	University of California Medical School
Alvarez, Walter C.	Mayo Clinic
Amberg, Samuel	Mayo Clinic
Amberson, W. R.	University of Tennessee Medical School
Amoss, Harold L.	Rockefeller Institute
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Anderson, H. H.	University of California Medical School
Anderson, John E.	University of Minnesota
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale University
Anderson, William E.	Yale University
Andrews, Edmund	University of Chicago
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.

Arnold, Lloyd	University of Illinois
Aronson, J. D.	Henry Phipps Institute
Asdell, S. A.	Cornell University
Asher, Leon	Berne, Switzerland
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Atchley, D. W.	Presbyterian Hospital, New York City
Atwell, Wayne J.	University of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis University
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Austin, W. C.	Loyola University
Avery, O. T.	Rockefeller Institute, N. Y. City
Babkin, Boris P.	McGill University
Bachem, Albert	University of Illinois Medical College
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bailey, Cameron V.	N. Y. Post-Graduate Medical School
Baitsell, George A.	Yale University
Bakwin, Harry	N. Y. University and Bellevue Medical College
Baldridge, Clarence W.	State University of Iowa
Baldwin, W. Manning	Albany Medical College
Ball, G. H.	University of California, Los Angeles
Banta, A. M.	Brown University
Banting, Frederick G.	University of Toronto
Barach, Alvin L.	College of Physicians and Surgeons
Barber, W. Howard	New York University Med. School
Barbour, Henry G.	Yale University
Bardeen, Charles R.	University of Wisconsin
Barlow, Orphus W.	Western Reserve University
Barnett, George D.	Stanford University
Barr, David P.	Washington University
Barron, E. S. G.	University of Chicago
Barth, L. G.	Columbia University
Bass, Charles	Tulane University
Bast, T. H.	University of Wisconsin
Bauer, J. H.	Rockefeller Foundation
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford University
Bayne-Jones, S.	Yale University
Bazett, H. C.	University of Pennsylvania
Beard, H. H.	Louisiana State Univ. Medical Center
Beck, Claude S.	Western Reserve University
Becker, E. R.	Iowa State College
Beckman, Harry	Marquette University Medical School
Beckwith, T. D.	University of California, Los Angeles
Behre, Jeannette A.	Cornell University Medical College
Belding, David L.	Boston University
Bellamy, Arthur W.	University of California, Los Angeles

Benedict, S. R.	Cornell University Medical College, N. Y. City
Benton, Anne G.	Minneapolis, Minn.
Berg, B. N.	Columbia University
Berg, William N.	New York City
Bergeim, Olaf	University of Illinois
Bergey, David H.	National Drug Co., Philadelphia
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Berry, George P.	University of Rochester Medical School
Beutner, R.	University of Louisville
Bieter, Raymond N.	University of Minnesota
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Bing, Franklin C.	Western Reserve University
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	Paris, France
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	University of California
Blackfan, K. D.	Harvard Medical School
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale University
Blakeslee, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blalock, Alfred	Vanderbilt University Medical School
Blatherwick, Norman R.	Metropolitan Life Insurance Co., New York City
Blau, Nathan F.	Cornell University Medical College
Blinks, L. R.	Stanford University
Bliss, Sidney	Tulane University
Block, Richard J.	Yale University
Block, Robert G.	University of Chicago
Bloom, William	University of Chicago
Bloomfield, A. L.	Stanford University Medical School
Bloor, W. R.	University of Rochester
Blum, Harold F.	University of California
Blumgart, H. L.	Beth Israel Hospital, Boston
Bodansky, A.	New York City
Bodansky, Meyer	John Sealy Hospital, Galveston, Texas
Bodine, J. H.	State University of Iowa
Bodo, Richard	N. Y. University and Bellevue Med. College
Bollman, Jesse L.	Mayo Clinic
Boor, Alden K.	University of Chicago
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Presbyterian Hospital, New York City
Borsook, Henry	California Institute of Technology
Bowen, B. D.	Buffalo General Hospital
Boyd, Theo. E.	Loyola University
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Bradley, H. C.	University of Wisconsin
Brand, Erwin	New York State Psychiatric Institute
Branham, Sara E.	National Inst. of Health, Washington
Brewer, Robert K.	Syracuse University
Briggs, A. P.	St. Louis University
Brodie, Maurice	Dept. of Health, New York City

Bronfenbrenner, J.	Washington University
Bronk, D. W.	University of Pennsylvania
Brooks, Clyde	Louisiana State University Medical Center
Brooks, Harlow	New York University
Brooks, Matilda M.	University of California
Brooks, S. C.	University of California
Broun, G. O.	St. Louis University Medical School
Brown, E. D.	University of Minnesota
Brown, J. Howard	Johns Hopkins University
Brown, John B.	Ohio State University
Brown, L. A.	Transylvania College, Lexington, Ky.
Brown, Rachel	N. Y. State Dept. of Health
Brown, Wade H.	Rockefeller Institute, N. Y. City
Buchanan, Robert E.	Iowa State College
Buchbinder, W. C.	Michael Reese Hospital, Chicago
Buell, Mary V.	Johns Hopkins University
Bulger, H. A.	Washington University
Bullowa, J. G. M.	Harlem Hospital, N. Y. City
Bunting, C. H.	University of Wisconsin
Bunting, R. W.	University of Michigan
Burch, John C.	Vanderbilt University Med. School
Burget, G. E.	University of Oregon Medical School
Burky, Earl L.	Johns Hopkins Hospital
Burns, Robert K., Jr.	University of Rochester
Burr, Harold S.	Yale University
Burrows, M. T.	Pasadena, Calif.
Byerly, T. C.	U. S. Animal Exp. Farm, Beltsville, Md.
Byrne, Joseph	Fordham University
Calkins, Gary N.	Columbia University
Cannan, Robert K.	N. Y. University and Bellevue Med. College
Cannon, Paul R.	University of Chicago
Cannon, Walter B.	Harvard Medical School
Carlson, A. J.	University of Chicago
Carmichael, E. B.	University of Alabama School of Medicine
Carruthers, Albert	Peiping Union Medical College
Carter, Edward P.	Johns Hopkins Hospital
Casey, Albert E.	Rockefeller Institute
Cash, James R.	University of Virginia
Cattell, McKeen	Cornell University Medical College
Caulfeild, A. H. W.	University of Toronto
Cecil, R. L.	Cornell University Medical College
Chace, Arthur F.	N. Y. Post-Graduate Medical College
Chaikoff, I. L.	University of California
Chambers, Robert	New York University
Chambers, William H.	Cornell University Medical College
Chang, Hsi Chun	Peiping Union Medical College
Chang, Hsiao-Chien	Peiping Union Medical College
Charipper, H. A.	New York University
Cheer, S. N.	Central Hospital, Nanking, China

Chen, K. K.	Eli Lilly and Co., Indianapolis
Cheney, R. H.	Long Island University
Chidester, F. E.	University of Michigan
Child, C. M.	University of Chicago
Chittenden, R. H.	Yale University
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	University of Michigan
Clark, A. J.	University of Edinburgh, Scotland
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	University of Wisconsin
Clarke, Hans T.	College of Physicians and Surgeons
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clawson, Benjamin J.	University of Minnesota
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Clifton, Charles E.	Stanford University
Coca, A. F.	Oradell, N. J.
Coghill, G. E.	Wistar Institute
Cohen, Barnett	Johns Hopkins Medical School
Cohen, Martin	N. Y. Post-Graduate Medical School
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Isidore	New Orleans, La.
Cole, Harold H.	University of California, Davis
Cole, L. J.	University of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Rutgers University
Coleman, Warren	New York University Medical College
Collens, William S.	Brooklyn Jewish Hospital
Collett, Mary E.	Western Reserve University
Collier, William D.	St. Louis University
Collins, H. H.	Crafton, Pa.
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